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ELECTROPHYSIOLOGICAL ABNORMALITIES DURING SLEEP IN SCHIZOPHRENIA: HIGH-DENSITY EEG STUDIES IN FIRST-EPISODE PSYCHOSIS AND FIRST-DEGREE RELATIVES

Tesi di Dottorato di Ricerca

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ABSTRACT

Schizophrenia (SCZ) is a heterogeneous syndrome with a chronic course that leads to relevant psychosocial impairment. The clinical phenomenology is typically defined by “positive” symptoms such as hallucinations and delusions and “negative” symptoms such as avolition and anhedonia, that are often associated with a progressive cognitive decline. Despite intensive research on the etiopathogenetic mechanisms of this disorder, clearly defined biological markers are still lacking. On the neurophysiological level, a marked reduction of sleep spindle activity has recently been described in patients with chronic, medicated SCZ and with early-course psychotic disorders. Whether such deficit can be considered a reliable marker of or an endophenotype of the disorder remains to be cleared.

The objective of my work was to accurately study the electrophysiological features of sleep spindle activity in First-Degree Relatives (FDRs) of SCZ patients and in drug-naïve subjects with First-Episode Psychosis (FEP). A high-density electroencephalogram (hd-EEG) with 256 channels was used to study a whole night of sleep in experimental subjects and in a sample of age- and gender-matched healthy control subjects. Several neurocognitive tests and clinical scales were also administered to FDR and FEP subjects in order define psychopathological status and vulnerability. None of the recruited subjects had received treatment with psychotropic medication.

Among several analysed parameters of sleep macrosructure and microarchitecture, hd-EEG measurements revealed a significant reduction of Integrated Spindle Activity (ISAs) in FDR subjects. ISA was obtained by integrating the absolute amplitude values of each spindle detected at every electrode, divided by its duration. No relevant differences were observed in terms of spindle density and duration.

Preliminary results from a small subgroup of drug-naïve FEP patients are also reported. The main findings of my work appear to support the view that sleep spindle deficit is an electrophyisological endophenotype of SCZ. Given the vast available knowledge on the cellular mechanisms underlying spindle activity, future perspectives include a molecular characterization in individuals who carry a known genetic vulnerability for thalamic dysfunction.

OUTLINE and MY CONTRIBUTION

PART I: this section provides a general introduction to the current diagnosis of Schizophrenia. After a brief historical note, the recent framing of nosographical criteria and fundamental clinical features of the disorder are presented.

PART II: in this chapter I briefly review the major empirical approaches that have been used to study the electrophysiology of Schizophrenia in wakefulness and during sleep. These include several recent studies conducted at our associate laboratory in the United States that began to characterize sleep spindle deficits in chronic, medicated patients. Many of the reported studies have been summarized in the recent publication: “Schizophrenia: from neurophysiological abnormalities to clinical symptoms.”

PART III: in this chapter I report the major production of my Ph.D. work, focused on the characterization of sleep spindle abnormalities in a population of First-Degree Relatives of patients diagnosed with Schizophrenia. This empirical approach is necessary to define the role of spindle deficits in the context of ongoing research. My findings seem to confirm the hypothesis that sleep spindles are an endophenotype rather than a clinical biomarker of the disorder. No definitive conclusions can be drawn from the second part of the chapter, in which very preliminary data are presented from a small group of drug-naïve, First Episode Psychosis patients.

PART IV: in this section I draw initial conclusions, describe ongoing studies and the possibility of deepening the level of investigation over the neurophysiological mechanisms described in the previous chapters with an ongoing *in vitro* study on reprogrammed neuronal cells of Schizophrenia patients and First-Degree Relatives at our associate laboratory in the University of Trento.

LIST OF PUBLICATIONS

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[BOOK CHAPTERS]

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D'Agostino A, Castelnovo A, Scarone S. "Non-pathological associations–sleep and dreams, deprivation and bereavement" In: The Neuroscience of Visual Hallucinations, 2014 59-89 John Wiley & Sons

LIST OF ABBREVIATIONS

AASM: American Academy of Sleep Medicine
APA: American Psychiatric Association
ARMS: At-Risk Mental State
BPRS: Brief Psychiatric Rating Scale
BS: Basic Symptoms
CAARMS: Comprehensive Assessment of at Risk Mental State
CASH: Comprehensive Assessment of Symptoms and History
DP: Dementia Praecox
EEG : Electroencephalography
EOG : Electrooculogram
EOS: Early-Onset Schizophrenia
ERP: Event-related Potential
FDR: First-Degree Relative
FEP: First-Episode Psychosis
fMRI : Functional Magnetic Resonance Imaging
HAM-D: Hamilton Depression Rating Scale
hd-EEG : high-density Electroencephalography
HR: High risk
ISAs: Integrated Spindle Activity
MRI : Magnetic Resonance Imaging
NREM: Non-rapid Eye Movement
PANSS: Positive and Negative Syndrome Scale
PFC: Prefrontal cortex
PV+: Parvalbumin positive
REM : Rapid Eye Movement
SCZ: Schizophrenia
TMS : Transcranial Magnetic Stimulation
TRN: Thalamic Reticular Nucleus
UHR: Ultra-High Risk
VEOS: Very Early-Onset Schizophrenia

SUMMARY

PART I

INTRODUCTION TO SCHIZOPHRENIA

1. DEMENTIA PRAECOX, OR THE GROUP OF SCHIZOPHRENIAS

1.1. Historical context

Schizophrenia (SCZ) is a heterogeneous disorder defined by a complex interaction of cognitive, behavioural and emotional dysfunctions. Despite intensive research in several fields, ranging from the genetic and biomolecular underpinnings of abnormal neural circuitry to neuropsychological and neurophysiological impairments, the core dysfunction remains elusive. The clinical phenomenology of this syndrome, originally termed Dementia Praecox (DP), was accurately studied at the turn of the 20th Century by Emil Kraepelin and Eugen Bleuler. These two authors clearly described signs and symptoms and defined the course and prognosis of the disorder (Bleuler, 1911; Kraepelin, 1913). The detailed analysis of psychopathology, clinical presentation, course and outcome yields a prototypical model characterized by an insidious onset and progression, with episodic exacerbations and a residual outcome in which affective blunting predominates. SCZ is commonly viewed as a neurodevelopmental disturbance coupled with a neurodegenerative process that begins during early adulthood. On the clinical level, it can be viewed as a syndrome with recurring episodes in which precocious cognitive decline holds a central role. Acute episodes of the disorder are typically described as re-exacerbations of psychotic symptoms such as hallucinations, delusions or disorganization.

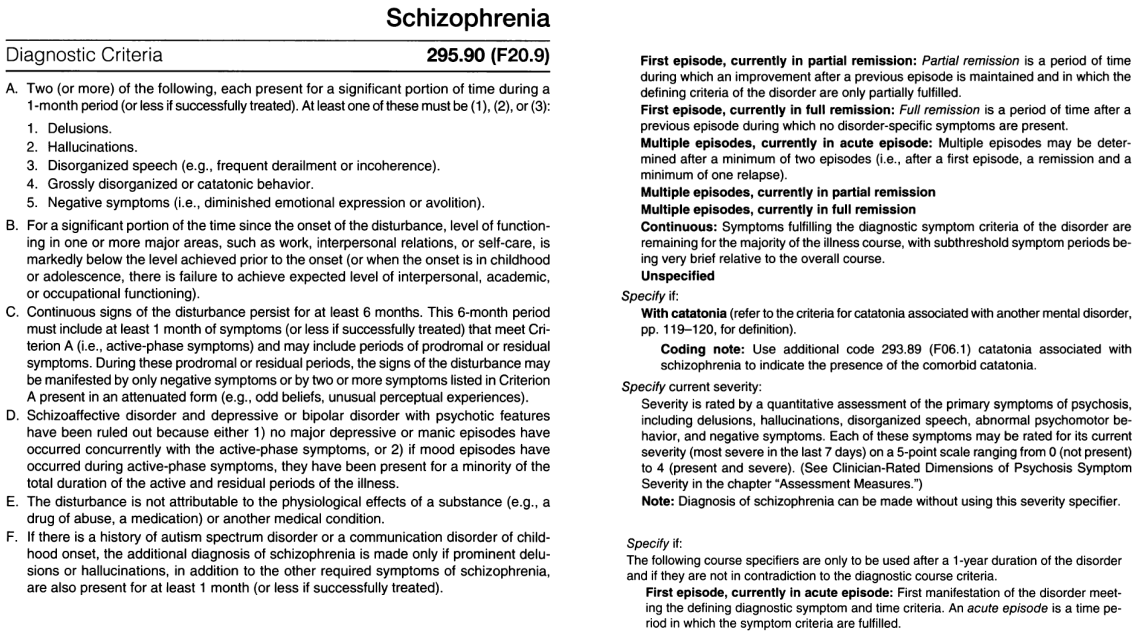
Bleuler's reconceptualization of DP was based on two fundamental clinical observations. In terms of evolution and outcome, not all DP patients appeared to evolve towards dementia and not all clinical conditions of DP had an early onset. In terms of core pathophysiology, the disintegration of subjectivity (defined as a unity of all Ego functions) was apparently shared by all patients. Supported by the experimental work conducted by Carl Gustav Jung at Burghölzli, Bleuler attempted to explain the disintegration of subjectivity through the clarification of psychological dynamics. Among others, his view that psychotic symptoms should be interpreted along a continuum with non-pathological phenomena and that neurophysiological and phenomenological features are shared with the experience of dreaming are still considered valid (Verdoux et al., 2002; D'Agostino and Scarone, 2013).

Bleuler was the first to explore pathogenetic mechanisms of the disorder, with the consequence of also proposing psychologically-oriented therapeutical perspectives. This led some authors to erroneously interpret his conception of the disorder as opposed to that of Kraepelin (Moskowitz and Heim, 2011; Fusar-Poli et al., 2008). Bleuler considered DP and SCZ synonyms that defined "a group of psychoses which develops at times in a chronic mode, at times by surges, which can stop or retrocede at any stage, but which probably doesn't allow a complete *restitutio ad integrum*" (Bleuler, 1911). Therefore, DP and SCZ identify the same disorder with an irreversible and non-periodic course. As far as aetiology is concerned, both Krapelin and Bleuler were convinced of the biological origin of the disorder, with the former oriented towards an exogenous, possibly toxi-infective insult, the latter more convinced of an endogenous,

primitive pathological modification of the brain. Our current understanding of the disorder confirms both authors had precociously hypothesized crucial mechanisms, with confirmed risk factors for SCZ ranging from genetic susceptibility to environmental insults (Meyer-Lindenberg, 2010).

1.2. Schizophrenia Spectrum in the DSM-5

From its third edition onwards, the Diagnostic and Statistical Manual of Mental Disorders (DSM) centred its clinical definition of SCZ on the transversal observation of psychotic signs and symptoms. This approach owes much to the conceptualization of the schizophrenic disorder operated by Kurt Schneider (Schneider, 1959), as explicitly recognized by some members of the American Psychiatric Association (APA) task force (Spitzer et al., 1978) In the latest edition of the DSM, Schizophrenia was conceptualized within a spectrum of diagnoses which includes Schizotypal Personality Disorder, Delusional Disorder, Brief Psychotic Disorder, Schizophreniform Disorder, Schizoaffective Disorder, Substance/Medication-Induced Psychotic Disorder, Psychotic Disorder Due to Another Medical Condition, Catatonic Disorders and other specified/unspecified Schizophrenia Spectrum Disorders (APA, 2013). DSM-5 diagnostica criteria for SCZ can be visualized in Figure 1.1



Schizophrenia

Diagnostic Criteria **295.90 (F20.9)**

A. Two (or more) of the following, each present for a significant portion of time during a 1-month period (or less if successfully treated). At least one of these must be (1), (2), or (3):
   1. Delusions.
   2. Hallucinations.
   3. Disorganized speech (e.g., frequent derailment or incoherence).
   4. Grossly disorganized or catatonic behavior.
   5. Negative symptoms (i.e., diminished emotional expression or avolition).

B. For a significant portion of the time since the onset of the disturbance, level of functioning in one or more major areas, such as work, interpersonal relations, or self-care, is markedly below the level achieved prior to the onset (or when the onset is in childhood or adolescence, there is failure to achieve expected level of interpersonal, academic, or occupational functioning).

C. Continuous signs of the disturbance persist for at least 6 months. This 6-month period must include at least 1 month of symptoms (or less if successfully treated) that meet Criterion A (i.e., active-phase symptoms) and may include periods of prodromal or residual symptoms. During these prodromal or residual periods, the signs of the disturbance may be manifested by only negative symptoms or by two or more symptoms listed in Criterion A present in an attenuated form (e.g., odd beliefs, unusual perceptual experiences).

D. Schizoaffective disorder and depressive or bipolar disorder with psychotic features have been ruled out because either 1) no major depressive or manic episodes have occurred concurrently with the active-phase symptoms, or 2) if mood episodes have occurred during active-phase symptoms, they have been present for a minority of the total duration of the active and residual periods of the illness.

E. The disturbance is not attributable to the physiological effects of a substance (e.g., a drug of abuse, a medication) or another medical condition.

F. If there is a history of autism spectrum disorder or a communication disorder of childhood onset, the additional diagnosis of schizophrenia is made only if prominent delusions or hallucinations, in addition to the other required symptoms of schizophrenia, are also present for at least 1 month (or less if successfully treated).

First episode, currently in partial remission: *Partial remission* is a period of time during which an improvement after a previous episode is maintained and in which the defining criteria of the disorder are only partially fulfilled.

First episode, currently in full remission: *Full remission* is a period of time after a previous episode during which no disorder-specific symptoms are present.

Multiple episodes, currently in acute episode: Multiple episodes may be determined after a minimum of two episodes (i.e., after a first episode, a remission and a minimum of one relapse).

Multiple episodes, currently in partial remission

Multiple episodes, currently in full remission

Continuous: Symptoms fulfilling the diagnostic symptom criteria of the disorder are remaining for the majority of the illness course, with subthreshold symptom periods being very brief relative to the overall course.

Unspecified

Specify if:

With catatonia (refer to the criteria for catatonia associated with another mental disorder, pp. 119–120, for definition).

Coding note: Use additional code 293.89 (F06.1) catatonia associated with schizophrenia to indicate the presence of the comorbid catatonia.

Specify current severity:

Severity is rated by a quantitative assessment of the primary symptoms of psychosis, including delusions, hallucinations, disorganized speech, abnormal psychomotor behavior, and negative symptoms. Each of these symptoms may be rated for its current severity (most severe in the last 7 days) on a 5-point scale ranging from 0 (not present) to 4 (present and severe). (See Clinician-Rated Dimensions of Psychosis Symptom Severity in the chapter "Assessment Measures.")

Note: Diagnosis of schizophrenia can be made without using this severity specifier.

Specify if:

The following course specifiers are only to be used after a 1-year duration of the disorder and if they are not in contradiction to the diagnostic course criteria.

First episode, currently in acute episode: First manifestation of the disorder meeting the defining diagnostic symptom and time criteria. An *acute episode* is a time period in which the symptom criteria are fulfilled.

Figure 1.1 Schizophrenia in DSM-5. Right column: Diagnostic criteria. Whereas no significant change was made in DSM-5 to criteria B and E, several changes were made to A and F. First of all, Schneiderian First-Rank Symptoms and bizarre delusions, previously considered pathognomic, have now been removed. Indeed, a growing amount of evidence appear to suggest these symptoms lack specificity, occurring as they do in other affective and non-affecitve psychotic disorders (Tandon, 2013). The presence of at least one positive symptom has also been introduced by DSM-5. Regarding Criterion F, in the previous version of the manual a diagnosis of SCZ could be made whenever hallucinations and delusions occurred in the presence of a previous history of autistic or pervasive developmental disorder. Given that other communication disorders exist with negative symptoms and disorganization at the onset (Dyck et al., 2011), the 1-month timeframe should allow a major diagnostic accuracy between SCZ and pervasive developmental disorders.

Finally, the major change was the loss of the four SCZ subtypes (disorganized, catatonic, paranoid, undifferentiated). This choice was sustained by two evidences: First of all, the distinction is nota t alla clear as the disorder progresses and, second, there doesn't appear to be a specific treatment choice for any of the subtypes. Only 5% of analysed studies found significant differences based on subtypes of the disorder, suggesting a lack of clinical and prognostic validity of such categorization (Braff et al., 2013). **Left column**: Course and severity specifiers. DSM-5 includes specifiers to identify the observed status (absent, partial or full remission from the acute phase) and the course of illness. These specifiers appear to be more valuable in directing a therapeutic approach based on the stage of the disorder and to be more accurate in prognostic terms at the level of single patients (Galderisi et al., 2013).

1.3. Clinical and epidemiological aspects of Schizophrenia

1.3.1 Epidemiology

SCZ is a relatively frequent disorder with an estimated world prevalence ranging from 0,3-0,66 to 1% and an incidence of 10,2-22 new cases per 100.000 persons/year (McGrath et al., 2008). However, data are often considered to be underestimated due to the unclear nosographical boundaries of the SCZ concept. Epidemiological data are mostly drawn from hospital registries so patients who never make contact with psychiatric services are difficult to estimate.

SCZ typically develops during late adolescence or young adulthood, peaking between 15 to 30 years of age. Incidence is higher in men, who also tend to have an earlier onset compared to women. Although rare, Early-Onset Schizophrenia (EOS) and Very-Early Onset Schizophrenia (VEOS) cases are reported in childhood, with a clear predominance of the male gender (McClellan e Stock, 2013). Typical aspects of the disorder overlap with the adult syndrome, but diagnosis is highly complex in children given that positive symptoms can be less elaborate and that visual hallucinations – easily confused with commonly reported fantasy play - tend to prevail. Furthermore, disorganization can be observed in many childhood onset neuropsychiatric syndromes and bizarre behaviour also occurs in children with Attention Deficit and Hyperactivity Disorder (ADHD). At the other end of the spectrum, the terms Late-Onset and Very Late-Onset Schizophrenia have been used for cases diagnosed after the

ages of 40 and 60. The majority of cases are women, with a predominance of psychotic symptoms with preserved affectivity and global functioning. Although criteria for the disorder are fullfilled, it remains unclear whether the pathogenetic mechanisms can be considered similar to those that are currently hypothesized to underlie conventional SCZ cases.

1.3.2 Course and outcome

The course of SCZ is highly variable, both inter- and intraindividually; symptom presentation and intensity, temporal evolution and remission rates vary considerably and are influenced by psychopharmacological interventions.
Some meta-analytic studies on the longitudinal course of the disorder found that up to 30% of patients treated at the First Episode had no re-exacerbations in the subsequent 5 years, whereas the majority of follow-up studies up to 40 years suggest that 20-70% of subjects have a chronic illness with re-exacerbations (Hegarty et al., 1994).

5-6% of subjects diagnosed with SCZ die by suicide, whereas up to 20% have a personal history of one or more suicide attempts. These can be secondary to hallucinatory commands including imperative voices that lead the subject to self-harm. Suicidal risk tends to remain high in both male and female subjects and is usually increased in those with Substance Use Disorder co-morbidity. Further risk factors for self-harm in SCZ are symptoms of depression / despair and the lack of a job; risk has been shown to increase during the period

immediately after a hospitalization caused by a psychotic re-exacerbation (Hegarty et al., 1994).

1.3.3 From the prodrome to Schizophrenia

The onset of SCZ is commonly preceded by a prodromal phase that can be characterized by mood, cognitive and attenuated psychotic symptoms. These are usually subthreshold in clinical terms and highly unspecific, so a clear distinction with temperamental traits or unusual subjective experiences of a healthy individual is highly complex.

The concept of a prodromal state, variously termed as *High Risk* (HR), *Ultra-High Risk* (UHR) or *At-Risk Mental State* (ARMS) was introduced to aid research and interventional programs in the early identification of SCZ. Several objective criteria have been proposed over time, although clear consensus remains to be reached. The “Basic Symptoms” (BS) concept is used to define disturbing subjective experiences that can be observed before and after a first psychotic episode (Gross, 1989). The *Brief Psychiatric Rating Scale* (BPRS, Overall and Gorham, 1962) and the *Comprehensive Assessment of Symptoms and History* (CASH, Andreasen, 1992) scales were used to measure the intensity of symptons, but a new scale was later developed to include an evaluation of duration and frequency, called *Comprehensive Assessment of at Risk Mental State (CAARMS,* Yung et al., 2005). BS appear to define an early prodromal stage and then wane, whereas CAARMS can detect later prodromal stages that are closer to the development of full blown psychotic symptoms.

High-Risk subjects do not appear as an homogeneous group and they can be divided in several subcategories as shown in Figure 1.2. Early identification of specific symptom domains in at-risk groups could lead to individualized treatment or follow-up programs to attempt a reduction in transition rates to SCZ (Fusar-Poli et al., 2013).

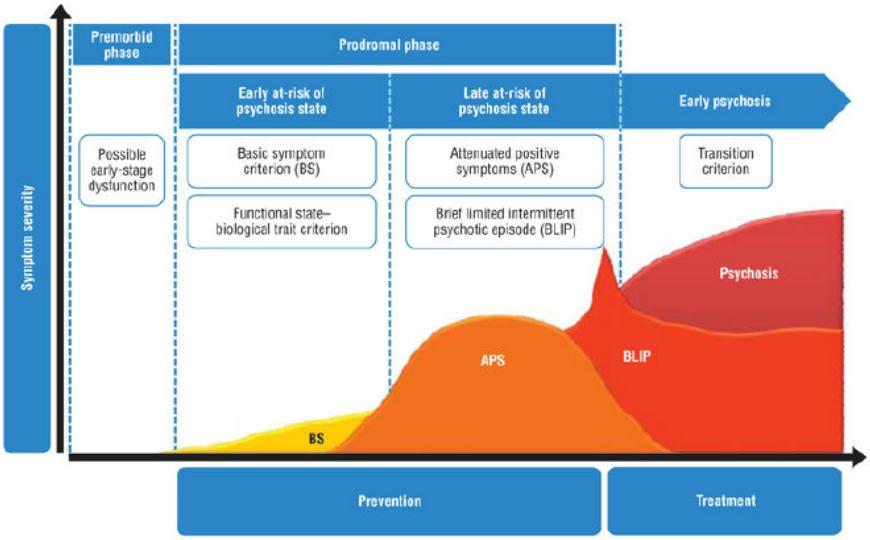


Figure 1.2. Prodromal stages of Schizophrenia. During the prodromal stage, subjects can be subdivided according to the following criteria (i) Attenuated Psychotic Symptoms (APS) that must have occurred in the previous year with attenuated intensity (APSa) or frequency (APSb); (ii) Brief Limited Intermittent Psychotic symptoms (BLIP), i.e. symptoms that resolve spontaneously within a year of their onset; (iii) subjects with a SCZ trait carrying a hypothetic vulnerability to psychosis, i.e. an affected FDR and with low sociofunctional status for at least a month of the previous year (Fusar-Poli, 2013).

1.4. Etiopathogenetic hypotheses of Schizophrenia

There are currently no radiological, laboratory or psychometric tests employed for the diagnosis of SCZ. Nontheless, differences are evident from neuroimaging, neuropathological, and neurophysiological studies in multiple brain regions between groups of healthy individuals and SCZ patients. Clear

differences have also been reported in cellular architecture, white matter connectivity and gray matter volume in a variety of regions such as the prefrontal and temporal cortices. Reduced overall brain volume has been observed, as well as increased brain volume reduction with age. Neurological “soft” signs including impaired motor coordination, sensory integration, and motor sequencing of complex movements; left-right confusion; disinhibition of associated movements are also commonly reported, along with minor physical abnormalities of the face and limbs. SCZ is considered close to autism spectrum disorders and neurodevelopmental disorders (Rapoport, 2009), with which genetic abnormalities and etiopathogentic pathways appear to be shared. SCZ is framed as a neurodevelopmental disturbance with multifactioral etiology, occurring in genetically vulnerable subjects on which multiple environmental and epigenetic factors trigger the onset of the disorder.

As far as genetic factors are concerned, approximately 80% of SCZ patients have a family history of the disorder, with a lifetime risk of developing the disorder in FDR of 6,5 % compared to 0,5% of the general population and 46% of subjects with both parents affected (van Os and Kapur, 2009). The major studies in this field included families of SCZ patients with mono- and dizygotic twins and adopted children, in order to discriminate between environmental and genetic impact.

1.4.1 Genetic models

Despite the evidence of a strong genetic influence on the development of SCZ, most affected individuals have no family history of any Psychotic Disorder. Liability appears to be conferred by a spectrum of common and rare risk alleles, with each contributing only a small fraction to the total population variance. Identified risk alleles are also associated with other major mental disorders, including Mood Disorders and Autism Spectrum Disorders. Indeed, a polygenic, multifactorial model is often used to explain SCZ (Haller, 2014). Several genes, many of which unknown, are thought to contribute a small increase of risk in carriers. The co-existence of multiple vulnerability polymorphisms within the same subject increases the risk of developing the disorder and combination with environmental factors leads to surpassing a susceptibility threshold thereby causing the disorder. Specific genes that have been most closely correlated with SCZ are Neuregulin 1 (NRG1), Dysbindin 1 (DTNBP1) localized on chromosome 6; DISC 1 (DISrupted in SChizophrenia) on chromosome 1 which appears to be involved in neuronal migration and arborization; genes coding for Catechol-O-methyl transferase (COMT), catecholamine-degrading enzymes that are also disrupted in the 22q11 deletion that determines De George syndrome, a disorder in which psychotic symptoms occur in up to 25% of subjects. However, the largest Genome-Wide Association Study (GWAS) conducted to date, identified 108 distinct genetic loci based on 36,989 patients (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014).

1.4.2 Environmental risk factors

The major environmental risk factors are obstetric complications and perinatal factors. Pre-eclampsia, Diabetes, Rh incompatibility, bleeding, abnormal fetal growth and development have all been associated with significant increases of risk to develop SCZ. Asphyxia, uterine atony and emergency Cesarean section have also been found to occur more commonly in the history of affected individuals. Hippocampal volume has been shown to be reduced in SCZ patients with a history of obstetric complications. Several animal studies have confirmed a strong relationship between intrauterine or intrapartum hypoxic insults and a vast array of developmental CNS abnormalities such as NMDA receptor reductions, increased DA release, white matter abnormalities, abnormalities of sleep/wake activation pathways (Schmitt et al., 2007; Mittal et al., 2008).

Increased paternal age and early exposure to various infectious agents also appear to confer a relevant risk increase for SCZ. Significant associations have been reported for Influenza viruses, Herpesvirus Simplex, Rubeovirus and Toxoplasma gondii. These agents have been hypothesized to interfere with neurodevelopmental trajectories directly by transplacental passage and indirectly by increasing proinflammatory cytokines produced during the maternal infection (Brown, 2011).

Major risk factors during adult life are physical and psychological stresses. The incidence of SCZ and related disorders is higher for children growing up in an

urban environment and for some minority ethnic groups. Cannabis use has also clearly been shown to increase the risk for psychosis and lead to increased rates of SCZ (Brown, 2011).

PART II

THE ELECTROPHYSIOLOGY OF SCHIZOPHRENIA

2. RECORDING BRAIN OSCILLATIONS

The electric activity of the brain can be measured across different frequency bands. Lower ranges include delta (<4 Hz), theta (4-8 Hz) and alpha (8-12 Hz), followed by high ranges beta (12-30 Hz) and gamma (>30 Hz) and ultrafast frequency ranges (>80 Hz) that have recently begun to gain more attention with a refinement and increase in sensitivity of available techniques. Oscillatory activity can be recorded at different levels, ranging to single cell potentials to complex neuronal activity inferred by measuring scalp potentials. Oscillations in humans are typically recorded with scalp electroencephalography (EEG), whereas depth electrodes implanted in neurosurgical patients have only recently employed to record oscillatory currents from subcortical structures. Extracellular local field potentials measure the sum of oscillatory activity from large groups of neurons. These extracellular currents are generated from a spatial addition of post-synaptic potentials of active cells. Indeed, EEG records the bioelectrical cerebral activity from the scalp and is a graphical representation of a difference in electric potential between two electrodes. The more neurons activate synchronously and with a parallel orientation, the higher the amplitude of the recorded signal.

Given the low conductivity of tissues, electrical currents attenuate with increased distance between the source and the recorder; furthermore, spatial diffusion and orientation of the recorder influence the reduction of voltage. The EEG signal is conducted by cables to amplifiers that are in turn connected to the acquisition and recording system.

3. EEG ABNORMALITIES IN SCHIZOPHRENIA

EEG recordings have been used extensively in SCZ research. Abnormalities such as an increase of power in low-frequency bands have been demonstrated throughout the course of illness, from the prodromal stage to chronic, medicated subjects. Several studies analysed the relationship between EEG parameters in various subtypes of SCZ, suggesting different topographical differences according to the specific subtype (Uhlaas and Singer, 2010). Despite such evidence the heterogeneity of the EEG picture has often been correlated to individual factors such as gender, age or ethnicity but also secondary and iatrogenic factors such as substance abuse or the long-term effect of psychopharmacological treatment. Technical aspects of recording such as open or closed eyes, the administration of cognitive tasks, the number and position of electrodes could also contribute to the variability across studies.

A growing amount of data suggest that cognitive dysfunction in SCZ patients is associated with abnormal synchronization of oscillatory activity in low and high frequency bands (Garakh et al., 2015). Several functional neuroimaging studies suggest abnormal coordination among distributed cortical regions during resting conditions that first appeared to only occur during cognitive tasks. Recent theoretical and experimental studies also reported that the functional coordination of such distributed regions is mediated by the synchronization among local oscillatory activities that are active at different frequency bands. Many known cerebral functions are associated with electrical activity at specific frequencies. Recently, the study of event-related potentials focused on the

gamma band given its prominent role in cognitive functions such as sensory elaboration, attention and memory; a growing number of studies proved that task-induced, regional gamma oscillations are reduced in SCZ pateints (Kikuchi *et al*., 2011; Moran e Hong, 2011). However, other studies had previously correlated different frequency bands with abnormalities of cognitive processing. Abnormalities of neural oscillations have also been shown during the resting state in SCZ. In 2008, a meta-analysis revealed an increase of slow rhythms in SCZ patients and a reduction of high frequency activity (Boutros et al., 2008). The analysed studies employed sophisticated strategies to control for biases such as ocular movements – which increase slow waves – and used various different experimental set-ups. Therefore, overlapping findings across studies confirm their validity. The most significant difference in SCZ patients compared to healthy control subjects, patients diagnosed with mood disorder or schizotypal traits was found in the theta and delta activity, which are increased in the frontotemporal regions of the brain (Garakh et al., 2015). The predominance of slow wave activity in frontal regions, with a reduction of blood flow and a consequent reduction of glucose is considered an electrophysiological correlate of hypofrontality in SCZ. Patients with an early onset of the disorder commonly have a higher power spectrum in slow rhythms and a reciprocal reduction of alpha rhythm; the increase of slow waves has also been found to be proportional to ventricle enlargement and negative symptoms. Subjects diagnosed with Schizoaffective Disorder share similar EEG patterns with SCZ patients, but the slow-wave increase more modest. Therefore, some authors suggested such feature should be considered a SCZ-specific EEG component compared to an “affective” alpha rhythm component (Schellenberg

et al., 1990). On the basis of such data, it seems plausible to conclude that an increase in delta (and – less convincingly – an increase of theta) could be a reliable marker of SCZ and that with adequate development this could become a valuable tool in the clinico-diagnostic domain. However, prolongation of recording times and standardization of artifact removal techniques to aid subsequent data analysis have been proposed to standardize methods and reduce heterogeneity of findings across studies (Boutros et al., 2008).

4. ENDOPHENOTYPES IN SCHIZOPHRENIA RESEARCH

4.1. What are endophenotypes?

The term endophenotype was introduced in the genetics of SCZ research as a measurable component, invisible to the eye, between genotype and clinical phenotype. Endophenotypes should be 1) associated with the clinical disorder but not part of its diagnostic criteria 2) heritable 3) independent of the state of illness, i.e. be present before the active pathology and/or after remissions 4) identifiable in unaffected relatives at a higher rate than the general population and it should 5) co-segregate with the disorder in families (Gottesman and Shields, 1973). The possibility of using an endophenotype as a prognostic marker is a further, highly desirable characteristic. The general idea that identification of the molecular bases is easier for endophenotypes than clinical phenotypes is broadly accepted, although genetic studies have been very few (Flint and Munafò 2007). Putative SCZ endophenotypes have been reported in several research domains, including but not limited to the fields of

neurophysiology (eyetracking dysfunction and abnormalities of electrophysiological indices such as PPI, P50, P300, N400), structural and functional neuroanatomy (cerebellar and olfactory tract abnormalities, reduced fronto-thalamo-cerebellar and fronto-striato-thalamic gray matter, cortical atrophy and abnormal development, enlargement of ventricles, hypofrontality), neuropsychology (reduced attention and vigiliance, working memory and executive deficits).

4.2. Neuroimaging Endophenotypes of Schizophrenia

HR subjects have been shown to have several abnormalties in terms of structure and volume of Gray Matter (Thermenos et al., 2013). The majority of available studies include structural or functional Magnetic Resonance Imaging, the latter usually associated with neuropsychological tasks across various different functions (attention, concentration, language, working memory); other studies employed DTI (Diffusion Tension Imaging) to analyze connections within white matter tracts. Despite several findings of regional volumetric reduction and disruptions of functional connectivity are commonly reported, data across different studies remain inconsistent. Major evidences regard the prefrontal cortex (PFC) as over half a dozen transversal studies found a volumetric reduction of such region whereas one longitudinal study found that HR subjects who went on to develop the illness had a global reduction of cerebral volume which was much more consistent in the PFC bilaterally when compared to those who had not illness. These patients also showed an

increase in the number of PFC gyri compared to those who did not develop the disorder (Harris et al., 2004).

From a functional perspective, a left hyperactivity correlated with abnormalities on cognitive testing, suggesting a possible compensatory effect of structural deficits for HR subjects to obtain an adequate functional status. Parieto-temporal structures also appear to be very engaged, with the Medial Temporal Lobe including the Hippocampus. Cortical thinning of these regions increases with time and correlates with severity of symptoms whereas fMRI studies showed increased activity of the left Medial Temporal Gyrus in those HR subjects who went on to develop the illness. Several studies found volume reductions of the amigdaloid-hippocampal complex and a functional reduction that was negatively correlated with verbal learning abilities (especially right parahippocampal) and cognitive function. Finally, some areas with less clear evidence of impairment were cerebellum and anterior cingulate cortex: the first was found to have a reduced volume, the second a reduced thickness especially in HR subjects with a higher genetic vulnerability and reduced function over several tasks (Thermenos et al., 2013).

Two aspects must be considered in the evaluation of these data: first of all, most available studies lacked a comparison between SCZ and other psychotic disorders and particularly affective psychoses. Second, contrasting results often emerge across groups. This appears to depend on the heterogeneity of the HR population. A clear example are fidings showing hyperactivity of certain structures in HR and hypofunction in ultra-HR subgroups. Age-related

differences have also been observed, possibly due to the elelvated development of local connections among close areas in children, which are progressively substituted with connections among more distributed areas during growth. Furthermore, some abnormalities in HR adolescents have been found to normalize at 17 – 20 years of age, suggesting a fundamental role for mean age of recruited subjects (Gogtay et al., 2007; Figure 4.1).

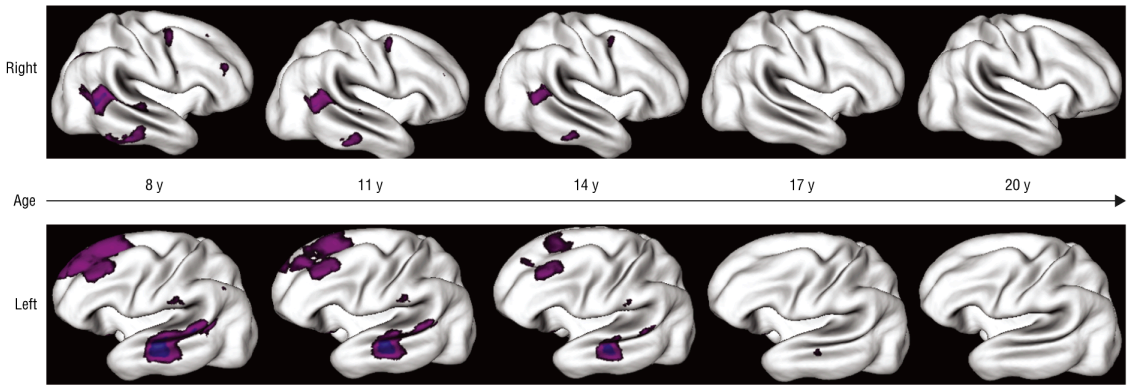


Figure 4.1. Cortical gray matter (GM) thickness of healthy siblings of patients with childhood-onset schizophrenia (COS) vs age–, gender– and scan interval–matched healthy controls. GM thickness in healthy siblings of patients with COS (n = 52; 113 scans) compared with healthy controls (n = 52; 108 scans) between ages 8 through 28 years. Healthy siblings show significant GM deficits in the left prefrontal and bilateral temporal cortices and smaller deficits in the right prefrontal and inferior parietal cortices. These deficits in healthy siblings normalize with age, with no abnormalities remaining by age 20 years. Gray matter cortical thickness is adjusted for mean cortical thickness (Gogtay et al., 2007).

4.3. Neurophysiological endophenotypes of Schizophrenia

Several EEG measures have been proposed as putative endophenotypes of SCZ. Studies can be substantially divided in those analyzing resting-state, spontaneous EEG, Event-Related Potential (ERP) studies and sleep EEG studies. One study of resting-state, spontaneous EEG including 128 SCZ

patients, 80 healthy relatives and 110 control subjects, reported a significant increase in gamma frequency band for patients but not for the other two groups, whereas reduction of theta and alpha rhythm were observed in patients and relatives (Hong et al., 2012).

ERP studies are based on continuous EEG recording during cognitive tasks. These studies indicate a modification of cerebral activity during a task. Responses are named with a letter (P for positive responses, N for negative ones) followed by the number of milliseconds elapsed from the stimulus to the event. The majority of ERP studies in SCZ can be divided into three categories. The first includes studies comparing patients with healthy control individuals; the second includes at-risk subjects in order to define the genetic susceptibity or endophenotype validity of a marker. The third category analyzes possible modifications of the marker during the course of antipsychotic treatment. One of the major ERP that has been identified as an endophenotype of SCZ is the P300. In a study designed to evaluate EEG during Clozapine treatment, P300 responses were studied pre-/post-treatment in 47 patients. A comparison with the EEG of 66 healthy volunteers indicated that several generators of P300 responses were modified after treatment and, furthermore, those of central and temporal regions had an 84% sensitivity in differentiating patients from control subjects (Ravan et al., 2012).

Along with P300, abnormal response to an auditory stimulus named Mismatch Negativity (MMN) has often been studied as a putative endophenotype. MMN amplitude is reduced in SCZ and several recent studies further validated this

hypothesis by demonstrating that MMN is already impaired in the early stages of the disorder and in subjects whose symptoms are attenuated below the threshold for a clinical diagnosis (Hasey e Kiang, 2013). Another ERP component that has been studied as an endophenotype of SCZ is the Error-Related Negativity (ERN), a negative ERP waveworm which peaks approximately 50 ms after a wrong response to a multiple response task. Although attenuated, this type of response can also be recorded when subjects are unaware of having made a mistake. ERN is reduced in SCZ patients as in ARMS and brothers of affected individuals.

P50 is an example of a possible SCZ biomarker. It is an early ERP response to auditory stimuli. When two stimuli are presented in rapid sequence, the second evoked P50 is physiologically inferior to the first, with its percentual reduction termed p50 suppression index. This suppression is diminished in SCZ and the entity of this decrease correlates with cognitive decline in patients (Potter et al., 2006).

Quantitative EEG (qEEG) during spontaneous resting-state has failed to show relevant differences in ARMS and in FEP subjects. On the other hand, such findings are common in chronic patients so they are likely to emerge in advanced stages of illness as a tardive sign of cognitive decline or possibly depend on pharmacological treatment, making them unlikely candidates as endophenotypes (Ranlud et al., 2014). Some data suggest that abnormalities are only present in HR subjects who went on to develop the disorder, whereas

the remaining did not differ from healthy control subjects (Bodatsch et al., 2011).

EEG sleep studies have only recently begun to emerge in the endophenotype literature. These will be analysed in major depth in the following section, given the specific focus of my work.

5. THE NEUROPHYSIOLOGY OF SLEEP

5.1. The Wake/Sleep Cycle

Sleep is thought to reflect a state of sensory disconnection from the environment and the reduction of functional cortico-cortical/cortico-thalamo-cortical connectivity is hypothesized to result in the restriction of consciousness and altered transmission of information within the brain (Tononi 2004). Sleep can be subdivided in Rapid Eye Movement (REM) and non-REM (NREM) sleep depending on the presence or absence of fast, intermittent ocular movements. The subdivision of sleep stages is based on polysomnographic recordings which combine electromyography (EMG) and electrooculography (EOG) to EEG. Despite the reduction in functional connectivity during NREM, the brain is still According to the latest AASM revision, NREM sleep is subdivided in three stages, each of which presenting its own features (Iber et al., 2007).

NREM Stage 1 (N1) is defined by low-voltage EEG with alternating alpha and theta frequencies, slow eye movements and preserved muscle tone. During this

stage vertex waves can be observed as brief, sharp transients with maximum negativity over central and – to a lesser degree – frontal midline derivations.

Theta and medium voltage delta waves are typical of NREM Stage 2 (N2). However, the stage is defined by the presence of K-complexes and sleep spindles. Whereas the former are bi- or triphasic elements characterized by a brief, negative component and a secondary slow wave, the latter are fusiform sinusoids that are most prominent over central midline regions. Their frequency is in the Sigma range (12-16 Hz) and they occur in short bursts of waxing and waning rhythmic activity with amplitude usually between 20 and 100 μV.

NREM stage 3 (N3) – often referred to as Slow-Wave Sleep (SWS), Delta Sleep or simply “deep sleep” – is defined by the presence of a slow, highly synchronous delta activity that can be observed in at least 20% of the recorded epoch. Spindles and K-complexes can still be seen, although Delta bursts, i.e. sequences of at least two high-voltage delta waves, are more typical of this stage.

REM sleep typically occupies 20–25% of total sleep and can be identified by REMs associated with loss of muscle tone and a fast, low-voltage, desynchronized EEG. Eye movements and muscle twitches correspond to the phasic stage of REM sleep as opposed to the intervening tonic stages. Combined transcranial magnetic stimulation (TMS)–scalp EEG techniques have recently confirmed a similarly widespread and differentiated pattern of cortical activation in REM sleep and wakefulness. This clearly distinguishes REM sleep

from other stages of sleep in which thalamocortical circuits progressively lose their capability of producing the complex responses to TMS observed in wakefulness (Massimini et al., 2010). Furthermore, phasic bursts have been shown to progress from the pontine nuclei to the lateral geniculate bodies and terminate with an activation of the occipital cortex. These currents of electrical activity, commonly referred to as ponto-geniculo-occipital waves (PGO) trigger a visual stream of information in the absence of external stimuli, and have as such been conceptually correlated to the emergence of the complex visual hallucinations found in dreams. Although typically found in REM sleep, they have been proposed amongst other overlapping phenomena to reflect a 'covert' REM sleep activation across other stages of sleep (Nielsen, 2005). According to this hypothesis, conscious subjective experiences outside of REM sleep can be explained by the co-occurrence of fundamental aspects of REM sleep during NREM periods.

Human sleep has long been considered a global phenomenon, coordinated by specialized and diffuse networks of neurons that modulate the activity of the whole-brain. However, depth recordings have recently begun to show that wakefulness and sleep are part of a continuum resulting from the complex interaction between diffuse neuromodulatory systems and intrinsic properties of different thalamocortical modules (Sarasso et al, 2014). The brain's continuous cycling between wakefulness and different stages of sleep has been proposed to reflect the balanced activity between the so-called 'REM-off cells' (serotonergic dorsal raphe nucleus and noradrenergic locus coeruleus neurons) and 'REM-on cells' (pedunculopontine tegmental neurons). REM sleep is

sustained by the activity of dopamine- and acetylcholine-releasing neurons and suppressed by the firing of noradrenergic, serotonergic and histaminergic neurons. The observed shift towards a highly active albeit closed system is cholinergically driven but also mediated by mesolimbic dopamine, which possibly contributes to the generation and motivational content of dreams.

Lack of coordination between activation and dampening of regulation systems might lead to the generation of dissociated stages of sleep (DSS), shared among physiological and pathological conditions. Intermediate Sleep (IS) is a transitional EEG pattern in which features of REM sleep are combined with k-complexes and spindles that typically define N2. IS represents 1-10% of physiological sleep in healthy subjects but it can increase in pathological conditions. More recently IS has been conceptually expanded to incorporate all those conditions in which different features of sleep are recorded simultaneously or with rapid oscillations; such patterns are grouped under the term DSS. These supposedly depend on abnormalities on various levels of sleep stage control systems so they can present as REM without atonia (RSWA), or without ocular movements (RSWR), or also as slow waves sleep with rapid eye movements (NRSWR) (Mahowald, 2011).

5.2. Physiological origin of Sleep Spindles

In humans, spindle activity is highly variable. A large number of studies observed two types of spindles which differ in terms of distribution and frequency; fast spindles are more easily observed in central and parietal regions

whereas slow spindles are typically recorded over frontal cortices bilaterally. The latter tend to occur after the former, although some overlap can be found. Furthermore, the majority of events appears to be limited to small cortical regions whereas global spindles are relatively rare. This theory was confirmed by a recent study in which a low correlation was found between cortical scalp-recorded spindles and hippocampal spindles recorded with depth electrodes in epileptic patients. Similar results had been reported from insular recordings. The authors suggest that spindle generation is under a strong local cortical influence, contributing to the view of sleep as a local phenomenon and challenging the view of sleep spindles as synchronous phenomena (Frauscher et al*.,* 2015).

Sleep spindles are generated in the thalamic reticular nucleus (TRN), a thin sheet of cells surrounding the anterolateral part of the thalamus (Halassa et al., 2011). The TRN is entirely composed of a rather heterogeneous population of parvalbumin immunoreactive GABAergic neurons (PV+) (Celio, 1990). The TRN is a thin thalamic nucleus which receives both thalamocortical and corticothalamic projections which contribute to its reticular aspect. The brainstem and forebrain also project to the TRN, through monoaminergic/cholinergic and GABAergic pathways respectively. GABAergic TRN neurons thythmically discharge on thalamic neurons which project to the cortex. This activity determines depolarization potentials across vast neuronal regions recorded on the scalp as spindles, high-frequency bursts that also induce a post-synaptic inhibitory potential in thalamic relay nuclei.

During wakefulness, the tonic activity of the TRN inhibits the background activity of thalamocortical relay nuclei, possibly under the influence of frontolimbic attentive circuits. TRN activity is also related to the “lateral” inhibition operated by stimulus-activated neurons which optimize response to sensory stimuli. Reduced efficiency of this system has been hypothesized to determine a reduction of signal-to-noise ratio, thereby impairing the precision of thalamic relay mechanisms (Ferrarelli e Tononi, 2011).

6. ABNORMAL SLEEP ELECTROPHYSIOLOGY IN SCHIZOPHRENIA

6.1. Sleep spindle deficit in Schizophrenia: State of the art

The study of sleep during EEG yields a unique window to study the physiology of brain function but also its pathology, given the abolition of wakefulness-related confounding factors such as fluctuating attention, diminishing motivation or cognitve faculties and interfering symptoms (Ferrarelli et al., 2007). Sleep/wake rhythm abnormalities or the architecture of sleep itself have long been recognized as a symptom of SCZ that can at times predate its onset. REM sleep abnormalities have often been reported, both in terms of latency and duration of single sleep cycles or total sleep duration. Furthermore, abnormalities of slow waves during sleep, reductions of N2, a general reduction of Delta rhythms and SWS deficit have all been reported across studies, although results tend to vary considerably (Gardner et al., 2014).

Spindle density and Integrated Spindle Activity (ISA) were recently found to be reduced on prefrontal, centroparietal and temporal regions (Ferrarelli et al., 2007 e 2010). The activity of spindles and slow wave activity was studied over a whole night of sleep in a group of 49 SCZ patients, comparing findings with the EEG of 20 subjects treated with antipsychotics for other psychiatric diagnoses (Major Depression, Bipolar Disorder, Post-Traumatic Stress Disorder, Panic Disorder and Generalized Anxiety Disorder) and 44 healthy control subjects.

SCZ subjects showed a decreased amplitude of spindles in centro-parietal regions and duration in prefrontal regions compared to control subjects. In this sample, slow waves rhythm was unchanged across the three populations. The amount of SWA is considered a reliable measure of brain function, given its independence from complex cellular interactions and the balance between excitatory and inhibitory mechanisms, both local and long-range. The amount of SWA during sleep has been found to be reduced in SCZ patients, although some findings suggest the opposite may be true (Keshavan,2011). Structural MRI and electrophysiological features were compared in SCZ and healthy control subjects, yielding a correlation between reduced volume in the mediodorsal thalamic nucleus (MD) and reduced number of recorded spindles in patients (Buchmann et al*.,* 2014). Furthermore, the origin of sleep spindle currents identified on the prefrontal cortex was found to be reduced and a correlation of the deficit with impaired performance on cognitive tasks suggest an involvement of thalamocortical circuit dysfunction in the cognitive decline that is commonly fobserved in SCZ patients (Buchmann et al*.,* 2014).

Sleep spindle deficits have been confirmed by an independent group which compared patients on antipsychotic treatment with different diagnoses and found impaired spindle activity only in SCZ patients (Wamsley et al*.,* 2012). A negative correlation was recently reported between spindle density and cortical Glutamate/Glutamine (Glx) levels measured with magnetic resonance spectroscopy in 20 healthy subjects (Figure 6.1 A). In the same study, a magical ideation scale was used as proxy for schizotypal personality traits, yielding an inverse correlation with spindle density (Figure 6.1 B) (Lustenberger et al., 2015).

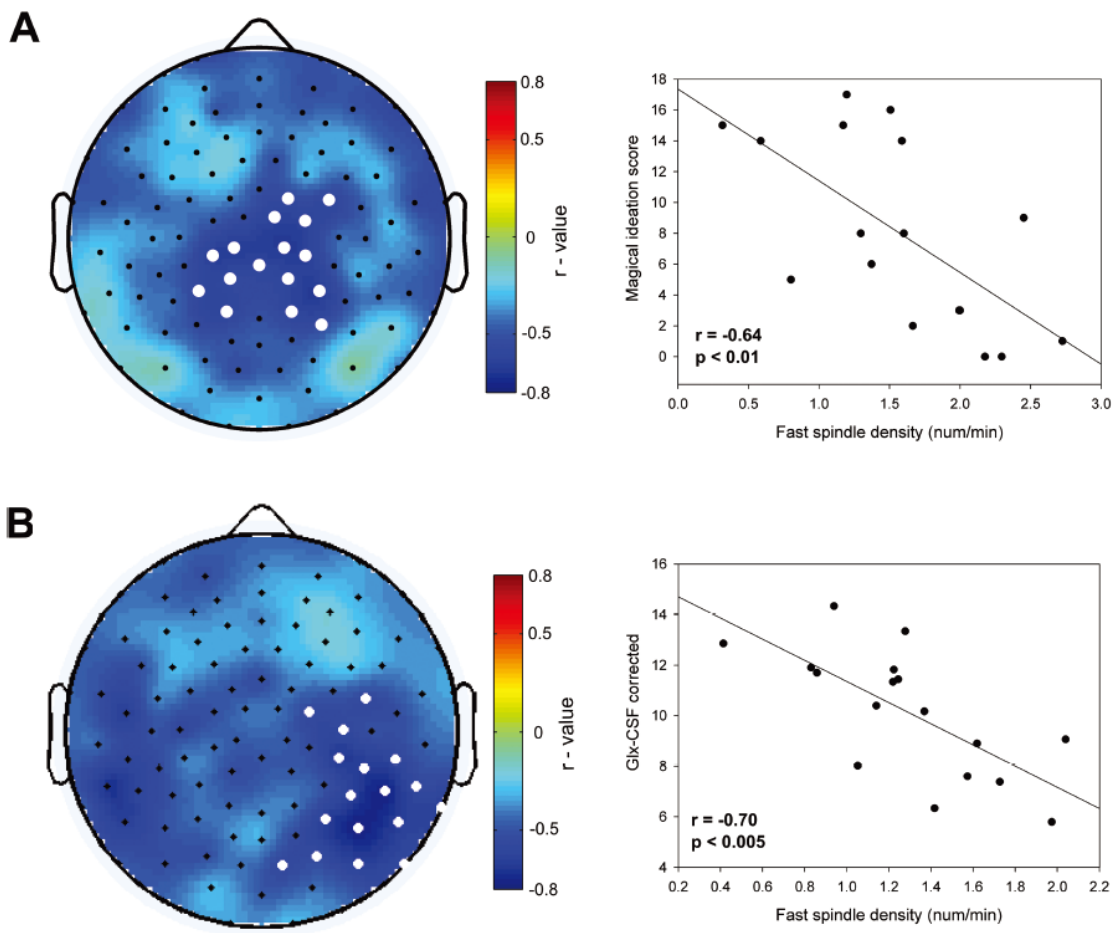


Figure 6.1 Correlation between fast spindle density (14.25–14.75 Hz) and (A) magical ideation score and (B) thalamic glutamine and glutamate levels corrected for CSF. Topographical distribution of r values is plotted on the planar projection of the hemispheric scalp model with negative correlations reflected in blue. White dots indicate significant electrode clusters after controlling for multiple comparisons. The scatter plots further demonstrate the significant correlation for the highlighted electrode clusters (Lustenberger et al., 2015).

One study recorded sleep EEG data of nine adolescents diagnosed with an Early Onset Schizophrenia (EOS) and compared data with a sample of age- and gender-matched healthy control subjects. Affected individuals had a low overall density that was more pronounced over centroparietal and temporal regions (see Figure 6.2). The deficit was marked in the 13,75 - 14,5 Hz frequency range. Furthermore, a negative correlation was observed between mean sleep spindle density and severity of positive symptoms. Therefore,

spindle density appears a useful candidate to aid the diagnostic process of SCZ, not only in adults but also in adolescent patients (Tesler et al*.,* 2015).

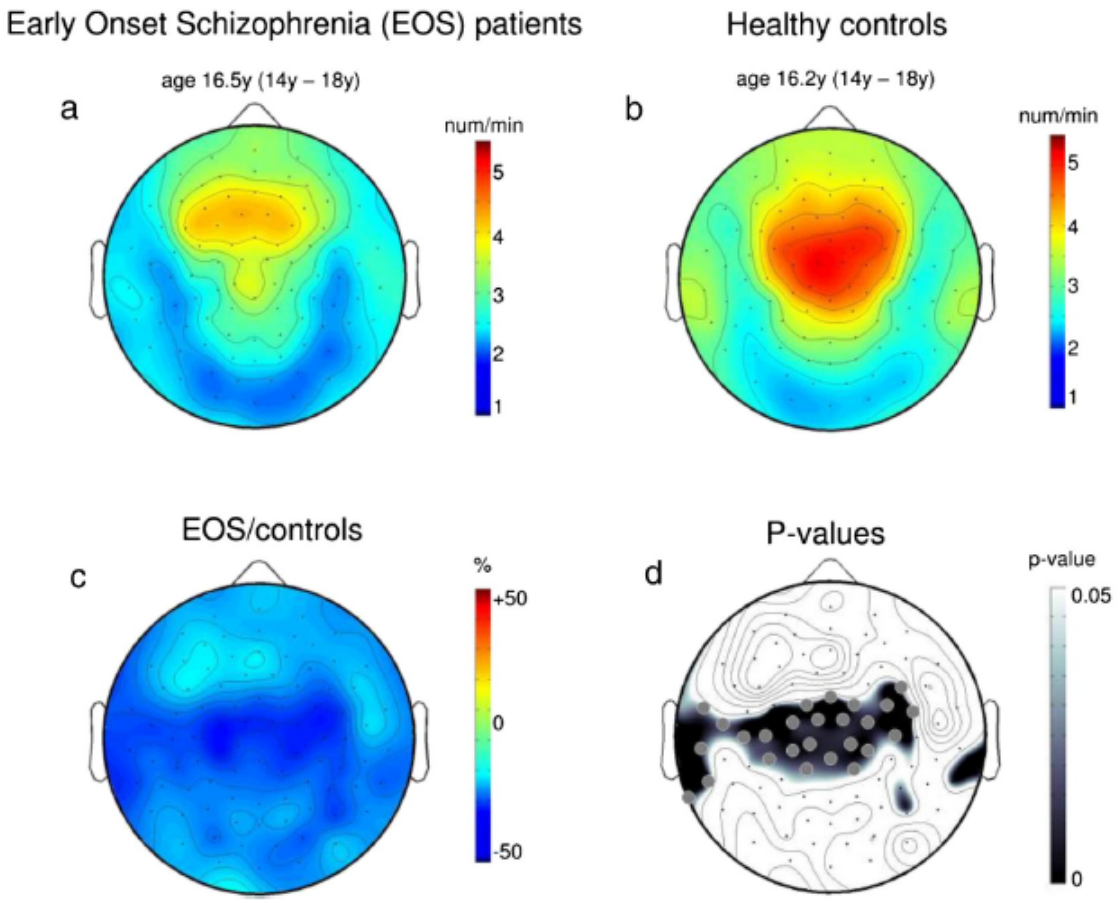


Figure 6.2 Topgraphic distribution of spindle density in patients with Early Onset Schizophrenia (EOS) and Healthy controls. The figure shows topographical distibution of spindle density during the first hour of NREM sleep for the 13.75 – 14 Hz frequency band in (a) 9 EOS subjects (b) 15 healthy controls (c) relationship between the two samples (d) corresponding statistically significant values.

Another study conducted on 16 chronic, medicated SCZ subjects confirmed a marked decrease of sleep spindles and impaired sleep-dependent enhancement of memory in affected individuals (Göder et al., 2015). These findings are in line with those of another group that recently reported significant

N2 spindle deficits in 15 newly diagnosed, antipsychotic-naïve patients with SCZ (Manoach et al., 2014). In this sample spindle activity correlated with positive symptoms and IQ estimates across groups.

However, one recent study on the sleep of 10 First-Episode, drug-naïve young adults diagnosed with SCZ failed to observe differences in spindle density. An excess of IS has been reported in several studies of SCZ compared to healthy control populations, although a recent study confirmed its presence but not at significant levels (Guénole et al*.,* 2014). The authors attribute such difference to the examined sample, given that drug-naïve patients had never previously been tested so observed abnormalities could be enhanced by medication. An increase of RSWA was first reported in this study, which had previously been reported in patients with Parkinson's Disease, Lewy Body Dementia, Narcolepsy and during treatment with antidepressant and hypnotic drugs.

6.2. Neurophysiological bases of observed abnormalities

The cerebral cortex is composed of 2 main types of cells: excitatory pyramidal cells which project to other parts of the cortex and non-pyramidal cells, including interneurons. These are small neural cells that communicate through inhibitory synapses and which are supposedly critical for the formation of oscillations.
GABAergic interneurons are usually connected to several pyramidal cells, creating multiple synapses with each and exerting a strong influence on their excitability. The coordinated modulation of membrane potentials by interneurons synchronizes pyramidal cells activity and the transformation of

electrical activity into oscillations: the longer the inhibition, the slower the oscillation. Over the past few years, the role of the thalamus in coordinating distributed cortical regions through rhythmic interactions has been progressively clairified. As an example, during visual attention processing in primates, the thalamic pulvinar nucleus coordinates activity in regions of the visual cortex by means of alpha band synchrony which is a performance-correalted physiological measure. Neuroanatomical imaging shows that the thalamus is organized in parallel pathways that independently reach the neocortex. Such property makes the thalamus a possible starting point for central abnormalities, leading to wide-scale brain effects from a single, restricted starting point (Cohen et al*.,* 2015).

GABA receptor dysfunction in SCZ patients is strongly supported and post-mortem studies reveal a loss of GABA markers in several cortical regions (Gonzalez-Burgos e Lewis, 2008). A specific subtype of GABAergic cells that are Parvalbumin-positive (PV+) appear to correlate with the production of gamma and theta frequencies (Moran e Hong, 2011). PV+ interneurons appear to be involved in various compex functions such as perceptive discrimination and attention and in the regulation of cerebral plasticity and in learning. The nature of identified abnormalities can vary from region to region and in various different subregions intraindividually. Disorders of GABAergic function could therefore casuse complex behavioural dysfunctions such as those observed in the highly variable syndromic presentation of SCZ patients (Benes et al*.,* 2015). One study revealed that the manipulation of a single receptor with substances

influencing interneuronal function could modifiy cerebral rhythms in a frequency-specific and region-specific way (Whittington *at al.,* 2011).

On a neuroanatomical level, many distinctive abnormalities of SCZ appear to contribute to impaired SWA, among which the most relevant are: enlargement of ventricles, a reduction of cortical thickness and a volumetric reduction of associative cortices and of the thalamus (Gardner et al*.,* 2014). Ketamine, a non-competitive NMDA receptor antagonist, can induce behavioral modifications that resemble SCZ, altered glutamatergic transmission can be hypothesized to reflect NMDA receptor blockade, of thalamic GABAergic interneurons and the TRN particularly, leading to disinhibition of cortical Glu release. Given that a neuronal subtype with specific biophysical properties can be found in this nucleus, hyperpolarization could determine its activation and increased EEG discharge recorded as delta band, the quantity of which has been associated to cognitive deficits in SCZ (Cohen et al*.,* 2015). Whereas psychopharmacological management of SCZ has commonly involved D2-receptor blockade, new studies on GABAergic and glutamatergic systems and reciprocal interactions may lead to the development of future therapeutic targets. One recent study (Wamsley et al*.,* 2013) found an increased spindle activity in SCZ patients after administration of eszopiclone, a GABAergic hypnotic. Other studies had previously shown an increase in spindle density and memory improvement in healthy volunteers, whereas benzodiazepine use leads to reduced SWR acting on GABA-A receptors (Gardner et al*.,* 2014). Multireceptor agents or specific polypharmacy could act by increasing the interneuronal discharge in order to specifically address only regions that are

functionally disrupted in the disorder. Along this line, glycine administration oe reduction of its synaptic reuptake by administration of a competitive antagonist such as bitopertin could improve negative symptoms, although results are contradictory (Cohen et al., 2015).

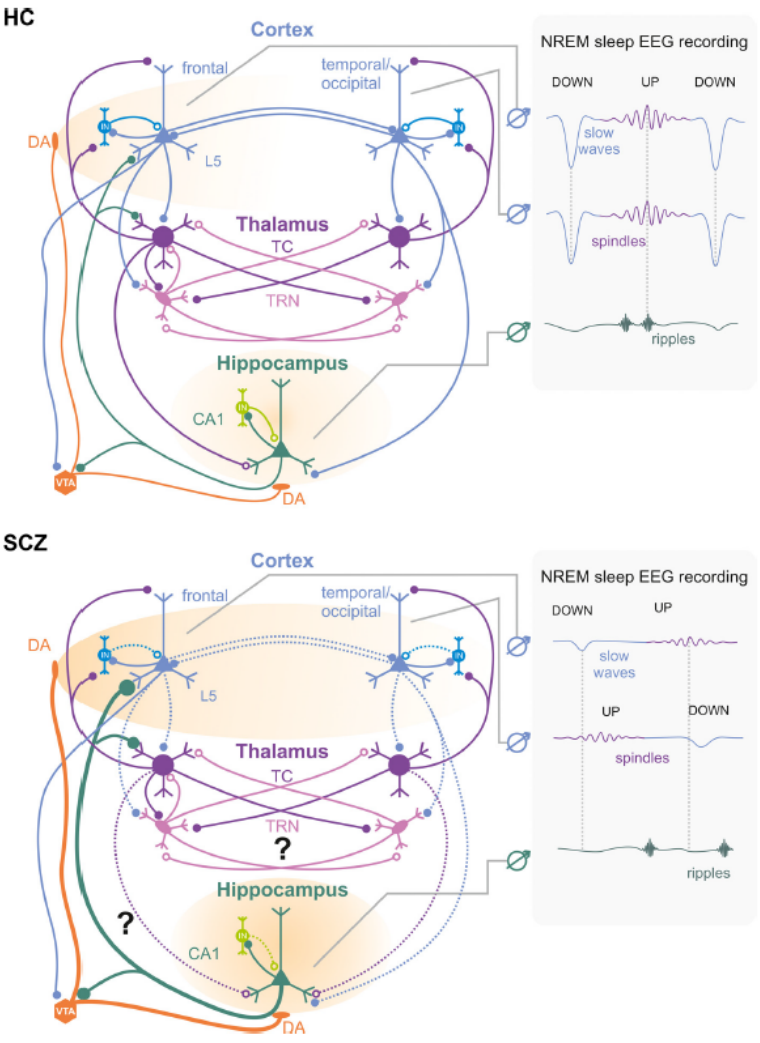


Figura 6.3 Circuits involved in the generation and synchronization of distributed networks during NREM sleep. The major circuits involved in the generation of NREM sleep include the Cortex, Thalamus and Hippocampus. Cortical and Hippocampal neurons are pyramidal cells of the deep player L5 and local interneurons (mainly PV+ basket cells). Thalamic neurons are thalamocortical cells and inhibitory cells of the TRN. **Top**: Sleep circuits in Healthy Control (HC) subjects. Cortical local currents in L5 and L6 deep layers are the major contributors to the generation of Slow waves (blue lines) Cortical efferences reach the Thalamus, where thalamocortical cells and the TRN generate spindles. **Bottom**: Sleep circuits in Schizophrenia (SCZ) patients, where cortical, thalamic and hippocampal abnormalities could influence neuronal activity during NREM sleep (Gardner et al., 2014).

PART III

HIGH-DENSITY EEG SLEEP STUDIES

7. SLEEP IN FIRST-DEGREE RELATIVES OF SCHIZOPHRENIA PATIENTS

7.1. Background

During sleep, the brain structures responsible for the gating of sensory information (thalamus) and the processing and response to input (cerebral cortex) are active and reciprocally communicating to produce the major spontaneous EEG oscillations of sleep: slow-waves and sleep spindles. These oscillations are a sensitive indication of the normal function of the brain and, since they occur during sleep, they do not require effort, attention or concentration from the subject. Sleep spindles, a defining characteristic of stage 2 sleep, are brief powerful bursts of synchronous 12–15 Hz neuronal firing in thalamo-cortical networks, which reach peak density late in the night (De Gennaro et al., 2000). Spindles induce massive influxes of calcium ions into cortical pyramidal cells, which are believed to trigger intracellular, calcium-dependent mechanisms required for synaptic plasticity (Sejnowski and Destexhe, 2000). Slow wave activity has been correlated with overnight improvement of motor procedural learning (Huber et al., 2004) and accumulating evidence suggests that sleep spindles mediate sleep-dependent consolidation of both procedural and declarative memory (Fogel and Smith, 2011). Our associate laboratory at the University of Wisconsin successfully identified reductions in both spontaneous EEG oscillations during sleep (sleep spindles) and reductions in functional connectivity (assessed by TMS-EEG during wakefulness) in subjects with schizophrenia (Ferrarelli et al. 2007; Ferrarelli et al. 2008). This deficit may reflect an abnormality in the function of a specific brain structure, the thalamic reticular nucleus, that is also involved with

regulating attention and brain processing of sensory information ("gating") during wakefulness. During sleep, the TRN plays a fundamental role in generating and maintaining spindles through cortico-cortical and cortico-thalamic loops. These findings are unique in the field and are particularly important as they implicate a specific brain structure and provide a readily measurable objective finding associated with illness. These preliminary findings have already been replicated by another group in unmedicated subjects with a recent diagnosis of any psychotic disorder (Keshavan et al., 2011), but mixed reports exist in the few studies on very small sample sizes which had previously reported increased (Hiatt et al., 1985) or unmodified (Van Cauter et al., 1991; Poulin et al., 2003) spindle counts when compared to healthy control subjects.

In order to clarify the role of sleep spindles and to validate this marker as a a putative endophenotype of the disorder, we conducted a single-site, controlled, un-blinded study to examine changes in the activity of the sleeping brain in First-Degree Relatives (FDR) of SCZ subjects.

7.2. Materials and method

7.2.1 Experimental sample

FDRs were recruited primarily by referral from patients or from their ill relative's physician. They were given an information sheet and clean copy of the Informed Consent form. After a complete description of the study, written informed consent was obtained. Healthy control subjects had no major medical,

neurological, psychiatric or sleep disorder. They were selected for age and gender to allow adequate pair matching with the FDR group.

7.2.2 Inclusion/Exclusion Criteria

Inclusion Criteria (all subjects): Ability to provide written consent prior to admission; Ability to understand and speak Italian; Age of ≥ 16 years and ≤ 65; good general health determined by the investigator on basis of medical history, physical and neurological exam; a first-degree relative (FDR) with a diagnosis of Schizophrenia.

Exclusion Criteria (all subjects): Diabetes requiring insulin treatment; A serious heart disorder; A diagnosis of cancer within the previous 3 years; Clinically significant abnormalities on pre-study physical exam or physician evaluation; Subjects who met available criteria for alcohol/drug abuse problems within the previous six months or were using illegal drugs. Subjects who regularly performed night or late evening shift work or who traveled with time zone shifts >3h in the 3 weeks prior to participation.

Additional Criteria for the healthy control group: Control subjects did not have a current or past diagnosis of any Sleep Disorder; Control subjects were good sleepers without insomnia or excessive sleepiness (based on clinical interview and sleep rating scales); Control subjects did not have a current or past psychiatric diagnosis OR any First-Degree Relative with a history of major psychiatric disorders; Comparison subjects were not taking any medication with

psychotropic effects (e.g. antipsychotics, antidepressants, anxiolytics, psychostimulants, antihistamines or beta blockers - based on subjective reports and clinician judgment).

7.2.3 Study procedure

All participants were interviewed to obtain a complete psychiatric, neurologic and medical history and to rigorously exclude any psychiatric diagnosis based on DSM-IV-TR criteria (APA, 2000). All participants completed a battery of tests, the results of which are reported along with demographic characteristics of the experimental sample in Table 7.1:

(i) The Brief Assessment of Cognition in Schizophrenia (BACS, Keefe et al., 2004), which provides a brief, reliable and valid test of global neuropsychological function that has already been used in research on SCZ endophenotype research (Hill et al., 2013). Tests included in the BACS are the following: list learning, digit sequencing, verbal fluency, token motor task, symbol-coding and Tower of London. Normative values have been established using the Equivalent Scores method in order to enable comparison with other neuropsychological tasks commonly used in the assessment of the Italian population (Anselmetti et al. 2008).

(ii) Pittsburgh Sleep Quality Index (PSQI, Buysse et al., 1989) and Epworth Sleepiness Scale (ESS, Johns et al., 1991) were employed to assess subjective sleep quality and the presence/absence of relevant disorders. Participants' self-

reports were compared with available normative values (PSQI good sleeper <5; ESS normal ≤10)

(iii) Peters et al. Delusions Inventory (PDI). This self-administered test is commonly used to assess psychosis proneness in the general population. Participants completed the 21-item Italian version (Preti et al., 2002). PDI items address unusual subjective experiences and beliefs in the general population with questions in dubitative form. The optimal cut-off point for psychotic subjects has been set at 8, and the test has been shown to reliably distinguish between subjects with psychosis and subjects with other forms of mental disorder such as anxiety spectrum disorders. Finally, participants completed a revised version of the Launay-Slade Hallucination Scale (LSHS–R, Vellante et al, 2012). The questionnaire is commonly used in research settings to assess the prevalence of hallucinatory experiences in healthy subjects.

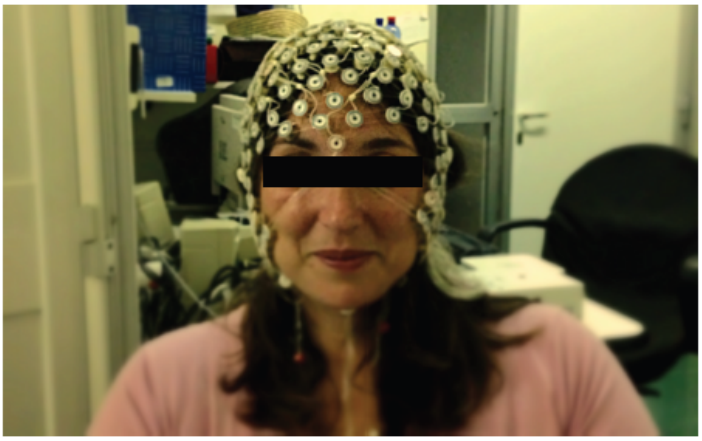
Characteristics	FDR	CONTROL
Age (years)	48.5 ± 14.2	49,8 ± 12.7
Gender (male : female)	8 : 8	8 : 8
Education (years)	13.8 ± 3.9	/
PSQI total score	3.3 ± 2.6	/
ESS total score	6.6 ± 2.7	/
LSHS-R total score	3.9 ± 5.4	/
PDI total score	2.9 ± 2.8	/
BACS list learning	49,6 ± 7	/
BACS digit sequencing	22 ± 3,2	/
BACS token motor task	67,3 ± 21,5	/
BACS verbal fluency	46,2 ± 14	/
BACS Tower of London	15,1 ± 4,7	/
BACS symbol-coding	52,5 ± 9,8	/

Table 7.1. Clinico-demographic characterization of the experimental sample

7.2.4 Sleep EEG recording

Patients were recruited at the Department of Mental Health of the San Paolo Hospital in Milan, Italy. EEG recordings took place in the same building at the Neurology II Unit - Regional Epilepsy Center - Sleep Medicine. All data were analysed at the Department of Clinical Sciences of the University of Milan.

Subjects were asked to arrive at the lab ~2 hours before their usual time of falling asleep. Whole-night high-density EEGs were performed with 256-electrode nets designed to improve electrode contact with the scalp, thereby enabling long-duration recordings (EGI, Eugene, Oregon, United States; Figure 7.1). After calibrating the equipment, standard baseline recordings were obtained while the subject was awake (resting wakefulness with eyes open and eyes closed). The subject was then accomodated in a sleep suite and allowed to sleep at the self-reported bedtime until morning.



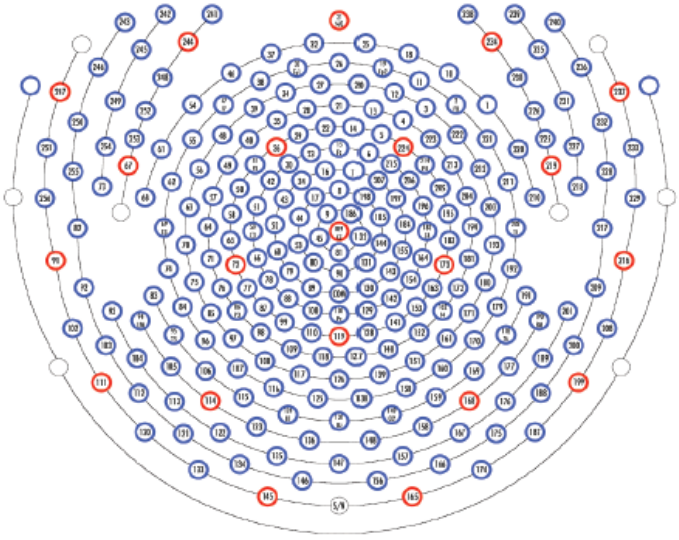


Fig. 7.1 High-density (Hd)-EEG montageis Dense-array scalp EEG recording systems consist of pre-cabled nets connected to a digital amplifier. The number of electrodes used can vary from 32 to 256 in commercially available nets. The high density of electrodes increases spatial accuracy, thus favouring an adequate, real-time source localization of the cortical signal, or detection of hidden EEG features. However, the relevant amount of data collected requires sophisticated post-processing analyses by experienced technicians to achieve significant results. Current clinical applications are limited to the precise presurgical identification of epileptic foci. Nontheless, the tool is suited for countless brain research applications, particularly in the field of sleep pathophysiology. Many studies have addressed specific sleep-related elements such as delta waves, spindles and K-complexes by means of hd-EEG, expanding the available knowledge over their physiological role (Pisarenco et al., 2014)

7.2.5 EEG data analysis

EEG signal was band-passed (0.5–50 Hz) and digitized at 500 Hz. RAW files were visually inspected in the EGI NetStation native software and then transferred to MATLAB (The MathWorks, Inc, Natick, Massachusetts, United States) environment for analysis. An 8-channel bipolar montage system was created for sleep staging [Right Eye Oculogram (REOG), Left Eye Oculogram (LEOG), F3-A2, F4-A1, C3-A2, C4-A1, O1-A2 and O2-A1]. I scored the data in 30 second epochs according to the American Academy of Sleep Medicine criteria, under the supervision of an expert (Iber et al., 2007). Alice Sleepware (Philips Respironics) was used and a csv file with scoring information was then created for use in Matlab, where the EEGlab toolbox (Delorme and Makeig, 2004) was implemented with customized scripts developed for this study. Clear artifacts were visually excluded from sleep staging during the scoring procedure.

A previously published artifact removal algorithm was used to reject 30-s epochs which exceeded thresholds based on the mean power for each channel in 0.8- to 4.48-Hz and 20- to 30-Hz bands (Ferrarelli et al., 2010). Signals were then re-referenced to the average for all included channels. Power spectral density of non-REM epochs was then computed with a 0.16 Hz bin resolution, fast-Fourier transform routine (Welch's averaged modified periodogram with a Hamming window, averages of five 6-second epochs). Approximately 1 – 3 undetected bad channels were then manually excluded for each subject after visual inspection.

An automated algorithm was then used to detect sleep spindles, as shown in Figure 7.2. Duration and amplitude of each detected spindle were measured. Spindle density and Integrated Spindle Activity (ISAs) were then calculated for each channel as follows:

(i) Spindle Density = number of spindles / minutes of NREM sleep

(ii) ISAs = Integration of absolute Amplitude value of each spindle / its duration

To characterize spindles in slow (12–14 Hz) and fast (14–16 Hz) frequency ranges, spindle parameters were then also calculated from NREM data filtered in these two frequency bands.

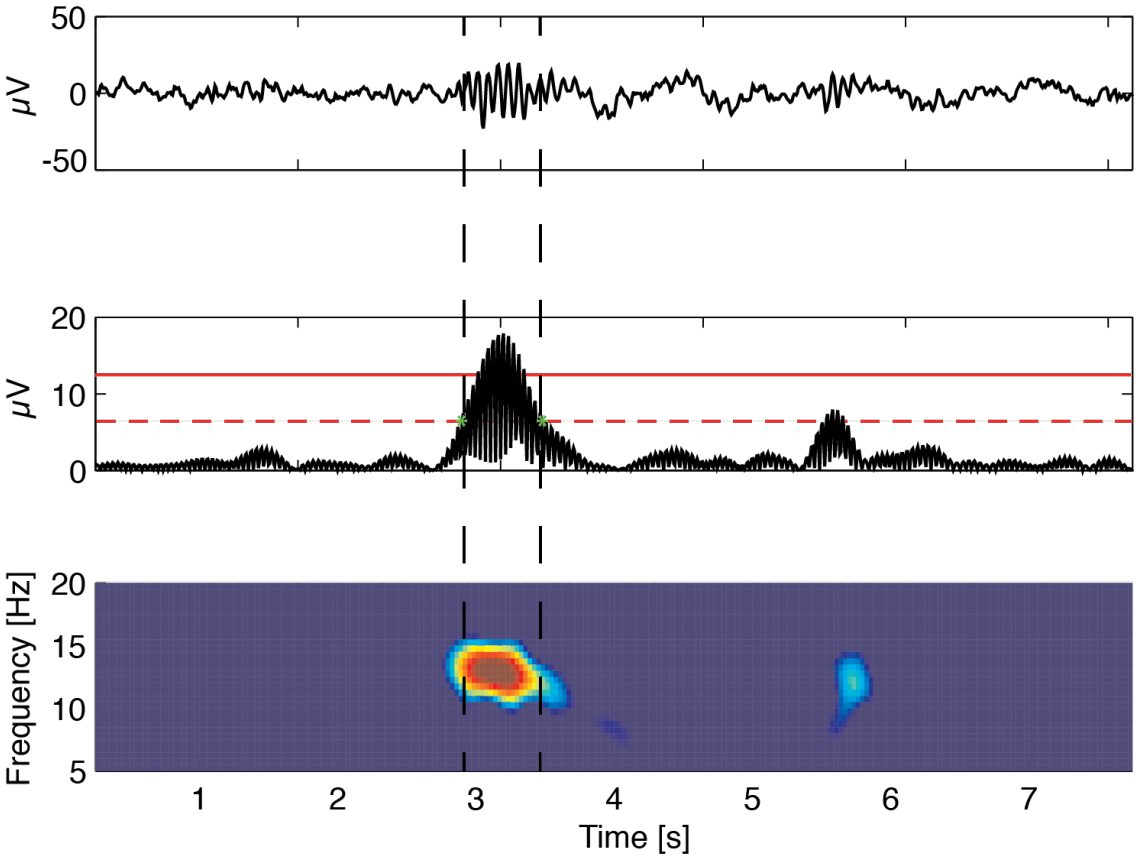


Figure 7.2 Spindle detection procedure. NREM epochs were band-pass filtered between 11 and 16 Hz and the amplitude of the rectified filtered signals were used as time series for each channel. Because signal amplitude varied significantly across channels, thresholds relative to the mean amplitude of each channel were used. The lower threshold was set at two times the mean amplitude of the channel signal and the upper threshold was set at eight times the mean amplitude. Whenever an amplitude fluctuation exceeded the upper threshold, a spindle was detected. Points preceding or following (≥0.25 second) this maximum when amplitude dropped below the lower threshold were considered as beginning and end. Green crosses indicated detected spindles; vertical dashed lines enclose detected spindles.

7.2.6 Statistical analyses

To compare demographic characteristics, sleep architecture, and EEG power between groups, unpaired t-tests were performed. Group differences in spindle density, duration and ISAS were assessed with Mann-Whitney U / Wilcoxon Ranks Sum tests. Cohen's d was also calculated for spindle parameters that differed between groups. Finally, we performed Spearman's rank correlation

analyses between spindle parameters and available neurocognitive tests and measures of psychosis proneness.

7.3. Results

7.3.1 Sleep Architecture

Sleep architecture differences between the two groups are shown in Table 7.2 Percentage of REM, N1 and N3 sleep differed significantly between groups. FDRs showed average 6% reductions of both REM and SWS sleep in favour of a > 10% increase of N1. Sleep efficiency was reduced by 9% in FDRs whereas TST presented a 25% reduction in FDRs compared to the healthy control group. Sleep latency, REM latency, WASO and N2 did not differ between groups.

Sleep variable	FDR	CONTROL	t-test	
			t	*p*
Total sleep time (min)	274,3 ± 77,3	368,1 ± 36,2	-4,390	0,0002**
Sleep latency (min)	30,8 ± 26,3	26,2 ± 8,8	0,662	n.s.
REM latency (min)	146,4 ± 64,2	117,5 ± 36,1	1,556	n.s.
Total waking time (min)	32,2 ± 13,5	23,1 ± 7,6	2,354	0,01*
Wake after sleep onset (min)	95,6 ± 36,9	86,3 ± 34,8	0,735	n.s.
Sleep efficiency	67,8 ± 13,5	76,9 ± 7,5	-2,354	0,01*
% Stage 1	14.2 ± 10,9	3,6 ± 2,3	3,821	0,001**
% Stage 2	51,7 ± 13,5	49,7 ± 7,8	0,651	n.s.
% Stage 3	19,4 ± 5,6	25,7 ± 8,7	-2,406	0,03*
% REM	14,6 ± 7,1	20,9 ± 3,8	-3,152	0,004**

Table 7.2 Sleep macrostructural differences between the two groups. *Significant values at $p < 0.05$ **Highly significant values at $p < 0.01$

7.3.2 EEG Power Analysis

EEG power during NREM sleep was calculated between 0.5 and 40 Hz for the two groups over the whole night and during the first cycle of sleep (Figure 7.3). Significant group differences in the spindle range were observed during the first cycle (12–16 Hz), with a nonsignificant trend emerging for the whole night.

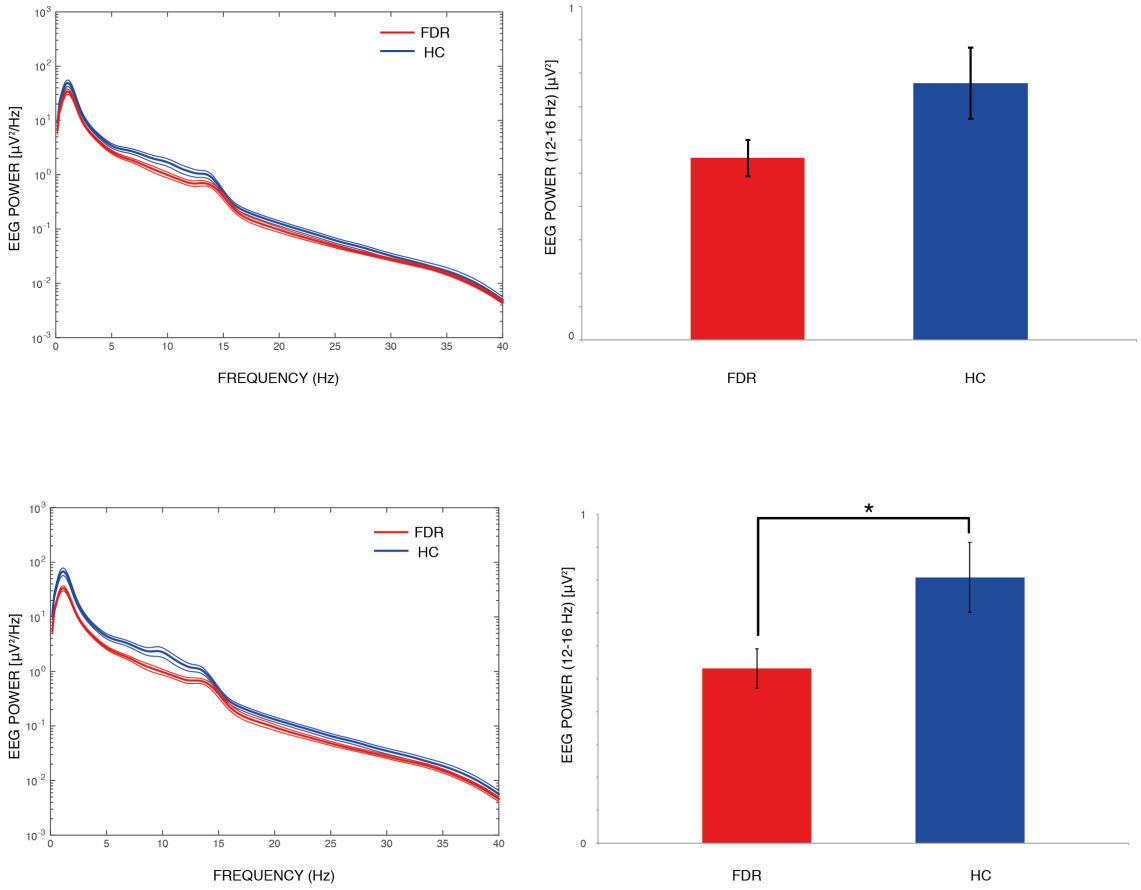


Figure 7.3 EEG spectral power plots in First-Degree Relatives (FDR) of Schizophrenia patients and Healthy Control (HC) subjects. Left: The plots show spectral power in the broad 0 – 40 Hz range over the whole night (top) and during the first cycle (bottom). **Right:** Mean group differences restricted to the sigma power range. Power deficits in FDRs were markedly visible during the first cycle (bottom) but they were not restricted to spindle range, given significant differences for frequencies <14 Hz.

7.3.4 Spindle Analysis

An initial analysis was performed for spindle density, duration and integrated activity in the 12–16 Hz frequency range for the whole night. The topography of each parameter was similar between groups, with peaks in prefrontal and centroparietal areas. Spindle density (Figure 7.4.1) and duration (Figure 7.4.2) in these regions largely overlapped between groups. No difference in slow / fast spindle duration or density was observed between the two groups.

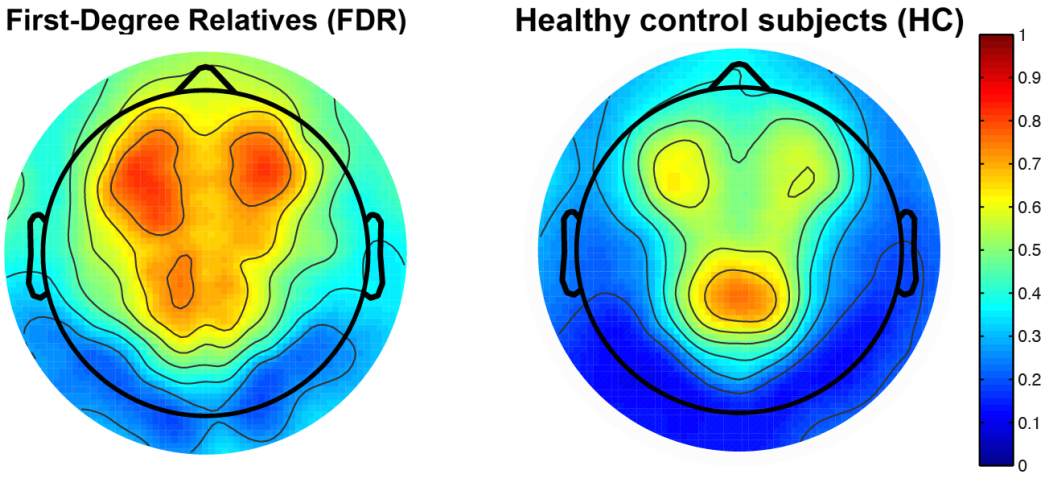


Figure 7.4.1. Whole-night sleep spindle density in First-Degree Relatives (FDR) of Schizophrenia patients and Healthy Control (HC) subjects. Topographical distribution of spindle activity in both groups confirms validity of the methodology employed. No statistically significant difference could be observed between FDR and HC groups in the whole sigma frequency range. Further analyses (topographies not shown) failed to detect significant between-group differences also for fast and slow spindle frequency ranges.

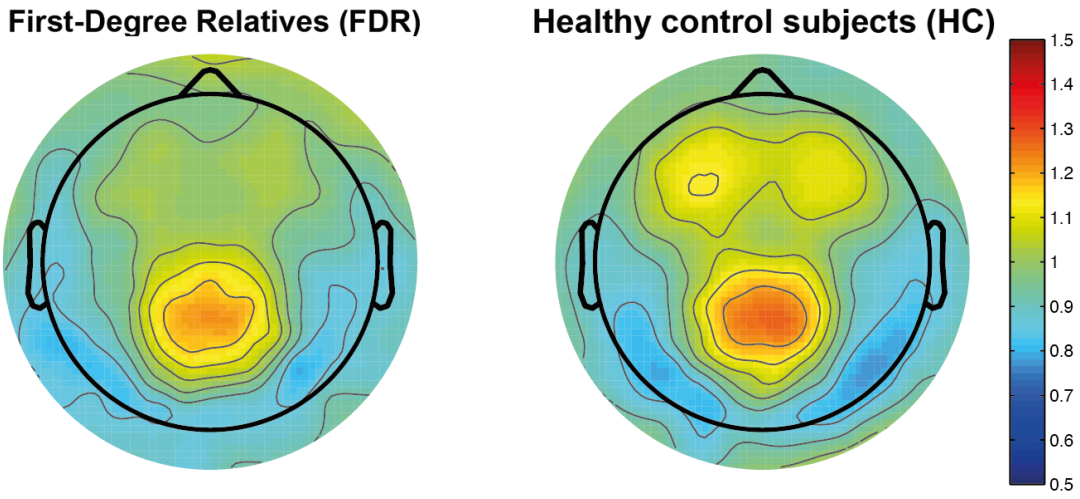


Figure 7.4.2. Whole-night sleep spindle duration in First-Degree Relatives (FDR) of Schizophrenia patients and Healthy Control (HC) subjects. No statistically significant difference could be observed between FDR and HC groups in the whole sigma frequency range. Further analyses (topographies not shown) failed to detect significant between-group differences also for fast and slow spindle frequency ranges.

A significant deficit of Integrated Spindle Activity compared to healthy subjects was clearly present in First-Degree Relatives (Wilcoxon Ranks Sum $p<0.05$) in prefrontal and centroparietal regions (Figure 7.5, top row). Additional analyses were performed for slow (12–14 Hz) and fast (14–16 Hz) spindles. Integrated Spindle Activity was significantly reduced in centroparietal regions for both frequency ranges (Wilcoxon Rank Sum, $p<0.05$).

Given the presence of significant power differences during the first cycle, all spindle parameters were then re-analysed but restricted to the first cycle. Again, topographical distribution of spindles peaked in prefrontal and centroparietal regions for both groups. Spindle density and duration overlapped between groups, yielding no significant difference. The significant ISAS deficit observed in the FDR group in the whole-night analysis was also confirmed for the first

cycle (Wilcoxon Ranks Sum $p<0.05$) in prefrontal and centroparietal regions (Figure 7.5, central row).

Finally, the same parameters were re-analysed but restricted to whole-night N2, the stage of sleep that is defined by the presence of spindles. ISAS was confirmed in the FDR group (Wilcoxon Ranks Sum $p<=0.05$) in prefrontal and centroparietal regions (Figure 7.5, bottom row). Additional analyses were performed for slow (12–14 Hz) and fast (14–16 Hz) spindles. Integrated Spindle Activity was significantly reduced in centroparietal regions for both frequency ranges (Wilcoxon Rank sum, $p<0.05$).

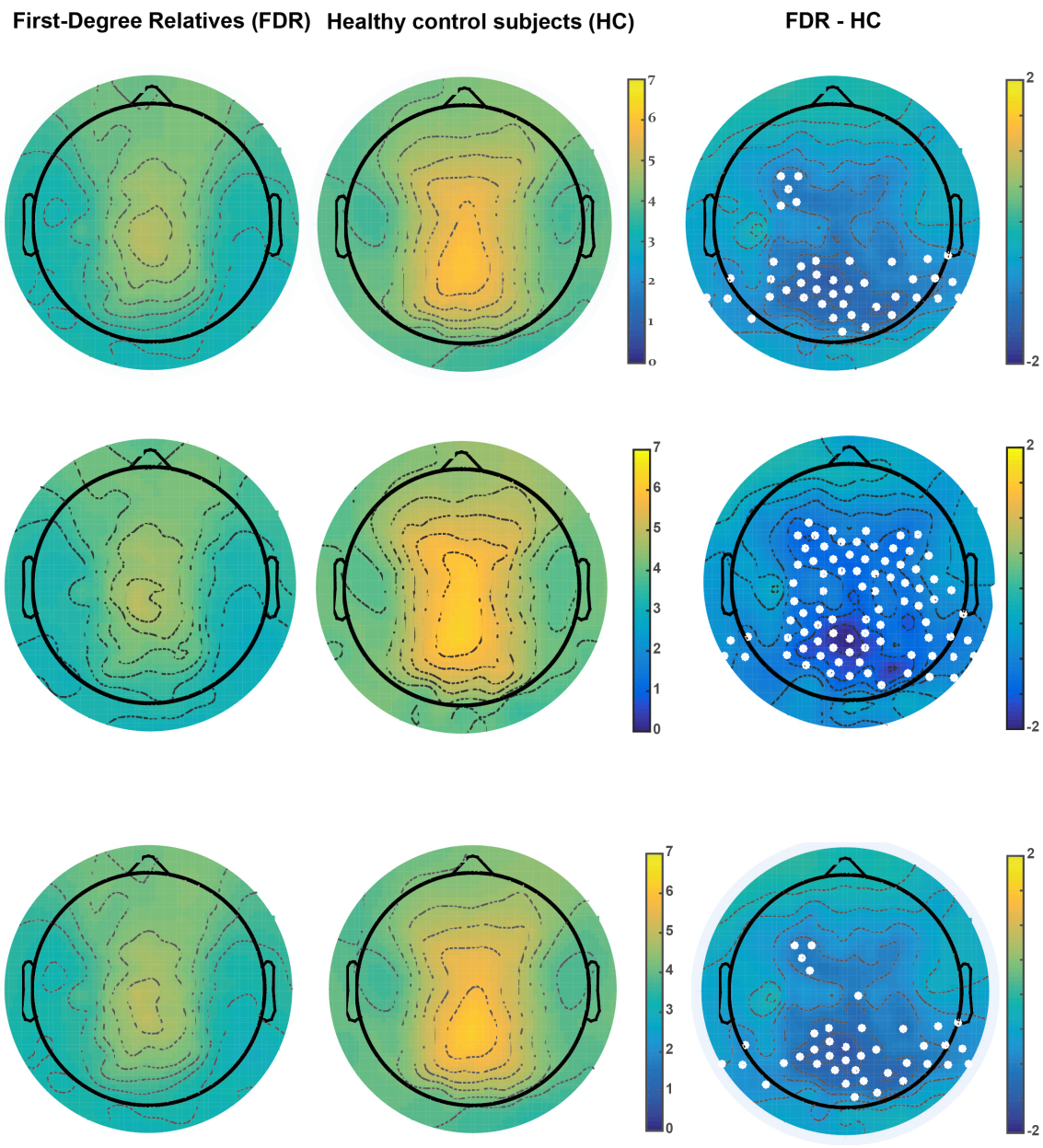


Figure 7.5. Integrated Spindle Activity deficit in First-Degree Relatives (FDR) of Schizophrenia patients and Healthy Control (HC) subjects. Significant differences were observed between FDR and HC groups in the whole sigma frequency range for ISAS. The strength of this finding was confirmed over the whole night (Top), during the first cycle (Centre) and also whole-night NREM stage 2 sleep (Bottom). Further analyses (topographies not shown) confirmed ISAS deficit in FDRs for both fast and slow spindle frequency ranges. White dots on right-sided topographies indicate electrodes showing significant differences between the two groups.

7.3.5 Effect Size of Integrated Spindle Activity deficit

In order to determine the magnitude of the observed spindle deficit in First-Degree Relatives, Cohen's d was calculated for ISAs as a measure of Effect Size. Cohen's d value ranged from an effect size equal to 0.99 for ISAs over the whole night to an effect size of 1.26 during the first cycle of the night. Cohen's d value was 1.1 and 0.8 for slow and fast spindles over the whole night and 1.33 and 1.1 respectively in the first cycle.

7.3.6 Measures of neurocognition and psychosis proneness

No significant correlation was observed between available neuropsychological tests and peak ISAs value. Spindle deficit also failed to yield any significant correlation with psychosis proneness scores in either the delusional thinking or hallucination scores. On the former scale, two subjects reached the lower threshold for psychsosis, whereas all others scored well below (Table 7.1).

7.4. Discussion of main findings

The main finding of this study is that First-Degree Relatives of subjects diagnosed with Schizophrenia have a partial deficit of Sleep Spindle Activity that can be summarized as a significant decrease of Integrated Spindle Activity with preserved physiology of Spindle Density and Duration. ISAs deficit was more pronounced during the first cycle of sleep and was confirmed for both fast and

slow spindle frequency ranges. To the best of our knowledge, this is the first study to evaluate this marker of thalamocortical function in a population of healthy, adult relatives of SCZ patients.

In terms of Sleep Architecture, compared to available normative values for the 40 - 60 years of age range (Ohayon et a., 2004), TST and REM% were the only parameters to be considerably reduced in the FDR sample. Indeed, Sleep latency was doubled and WASO was over three times the normative value (below 30 minutes) for both groups. Likewise, sleep efficiency was lower than expected (> 80%) for both groups. N1 was particularly reduced in our control population but close to norm in the FDR group. N3 was slightly increased in both samples and significantly lower in FDRs. This confirms one previous study in which SWS latency and duration variables were found to be reduced in SCZ patients and FDRs compared to healthy controls (Sarkar et al., 2010). N2 – the most relevant macrostructural value given the focus of this study on spindles – was in line with normative parameters and did not differ between groups.

The possible effect of sleep architecture on our main finding was ruled out with adjunctive analyses on N2 and on the first cycle of sleep. Indeed, the most conspicuous effect of ISAS difference between the experimental samples was observed during the first cycle of the night. Here, the deficit was widespread whereas it was more specifically localized on centroparietal cortical regions on whole-night NREM and N2 analyses. Although age- and gender-matched SCZ subjects were not directly compared in this analysis, previous studies at our associate laboratory revealed that patients have marked deficits in fast spindle

duration (prefrontal), amplitude (centroparietal), number and integrated activity (both prefrontal and centroparietal) that appear to be independent of medication.

Sleep spindles have been associated with neurocognitive performance in several studies (Keshavan et al., 2011; Wamsley et al.; 2012; Göder et al., 2015). No relevant correlation was found in our sample of FDRs between ISAS and adjusted scores on the cognitive battery. Significant impairment of BACS scores had previously been reported in a large sample of SCZ relatives without a history of psychosis (Hill et al., 2013). However, when compared to available normative values (Anselmetti et al., 2008), our FDR sample did not show relevant impairment in most subtests. Whereas motor task results were below the threshold for pathological performance, all tests of memory, verbal fluency and executive function were within median values.

To the best of our knowledge, only one study previously reported findings from the sleep of healthy, adult FDRs but only sleep architecture was addressed and no data on sleep spindle activity were reported (Sarkar et al., 2010). In the only previous study reporting sleep spindle activity of FDRs, recruited subjects were 17 children and 2 siblings of patients with confirmed diagnoses of SCZ (Manoach et al., 2014). Almost 70% of these children (mean age 14 ± 4) had a lifetime history of other major mental disorders: Attention-Deficit/Hyperactivity Disorder (n = 5), Major Depression (2), Separation Anxiety Disorder (2), Oppositional Defiant Disorder (2) and Conduct Disorder (2). Furthermore, at the time of the study one child was taking amphetamine/dextroamphetamine and

another was taking sertraline. This type of FDR sampling is likely to be more informative about ultra-high risk than strictly endophenotype research, which should focus on subjects with shared liability who do not develop a disorder. Furthermore, even severe abnormalities of cortical thickness in siblings of VEOS have been found to normalize by the age of 20, suggesting that children and adolescent data cannot be easily bridged with findings in adults (Gogtay et al., 2007). Finally, the authors of the study acknowledged that the observed deficit might have been influenced by the relatively low mean IQ level found in their young population. Indeed, IQ has been found to correlate with sleep spindles (Fogel and Smith, 2011). Although IQ was not specifically tested in our sample, the general quality of the neuropsychological performance might be considered a surrogate for a normal IQ range. From this viewpoint, one might speculate that multiple factors contribute to quantified spindle activity, so that more pronounced deficits are found in samples in which low IQ and genetic vulnerability for SCZ are combined.

ISA has been found to negatively correlate with positive symptoms and to clearly distinguish SCZ patients from both healthy subjects and patients with other major psychiatric diagnoses, making it a putative biological marker of the disorder. In terms of underlying pathophysiology, diffuse spindle deficits of SCZ patients have been attributed to dysfunction of the TRN spindle “generator”. Given the preserved physiology of spindle density and duration, the amplitude deficit observed in FDRs could reflect a partial dysfunction that does not impact on spindle number and duration but only size. On the other hand, smaller spindles could reflect a more general problem of thalamocortical connectivity,

leading to a recruitment of fewer thalamocortical fibers or an incomplete synchronization of cortical neurons which determines a discharge over relatively limited regions.

8. SLEEP IN DRUG-NAÏVE, FIRST-EPISODE PSYCHOSIS PATIENTS

8.1. Background

The thalamus has been proposed to play a major role in SCZ pathophysiology. One recent study confirmed several abnormalities of thalamic connectivity and began to clarify their relevance for several cerebral functions (Cheng et al., 2015). In HR samples, thalamocortical connectivity deficits appear to predict the development of full-blown psychosis. Furthermore, the observed hyperconnectivity between thalamus and prefrontal cortices, sensorimotor areas and the cerebellum appear to correlate with symptom severity (Anticevic et al., 2015). Sleep spindle deficits have been proposed to reflect aberrant thalamocortical connectivity given the thalamic origin of spindles (Ferrarelli and Tononi, 2011). However, most findings have been observed in chronic, medicated samples of SCZ patients and the characteristics at illness onset remain unclear. I report the preliminary findings from a subsample of drug-naïve subjects recruited during First-Episode Psychosis (FEP).

8.2. Materials and method

8.2.1 Experimental sample

FEP patients were recruited during their first hospitalization. After a complete description of the study, written informed consent was obtained. Healthy control subjects had no major medical, neurological, psychiatric or sleep disorder. They were selected for age and gender to allow adequate pair matching with the FEP group.

8.2.2 Inclusion/Exclusion Criteria

Inclusion Criteria (all subjects): Ability to provide written consent prior to admission; Ability to understand and speak Italian; Age of ≥ 16 years and ≤ 40; good general health determined by the investigator on basis of medical history, physical and neurological exam.

Refer to Paragraph 7.2.2. for Exclusion Criteria and Additional Criteria for the healthy control group.

8.2.3 Study procedure

FEP patients followed the same experimental procedure decribed in chapters 7.2.3 → 7.2.6. All participants completed a battery of tests, the results of which are reported along with demographic characteristics of the experimental sample in Table 8.1:

In this population, the following clinician-administered psychopathological rating scales were added to the evaluation:

(i) The Positive and Negative Syndrome Scale (PANSS, Kay et al., 1987) was used to evaluate the presence and severity of typical SCZ symptoms. It is composed of 30 items, 7 of which for positive symptoms, 7 for negative symptoms, 16 for general psychopathology.

(ii) The 21-item Hamilton Rating Scale for Depression (Ham-D, Hamilton, 1967) was used to quantify depressive symptoms.

Characteristics	FEP	CONTROL
Age (years)	24,4 ± 3,6	24,3 ± 3,4
Gender (male : female)	5 : 0	5 : 0
Education (years)	13.8 ± 3.9	/
PSQI total score	6.6 ± 2.7	/
ESS total score	3.8 ± 3.5	/
LSHS-R total score	21.8 ± 11.4	/
PDI total score	11.4 ± 4	/
PANSS total score	83.4 ± 13,4	/
HAM-D total score	12,4 ± 8,1	/
BACS list learning	40,2 ± 6,2	/
BACS digit sequencing	22 ± 3,2	/
BACS token motor task	58,4 ± 14,3	/
BACS verbal fluency	46 ± 18,7	/
BACS Tower of London	15,4 ± 6,2	/
BACS symbol-coding	41,8 ± 8,1	/

Table 8.1. Clinico-demographic characterization of the experimental sample

8.3. Preliminary Results

8.3.1 Sleep Architecture

Sleep architecture parameters in the two groups are shown in Table 8.2. The only significantly different value was the percentage of N3, which was diminished by roughly 9% in FEP patients.

Sleep variable	FEP	CONTROL	t-test	
			t	*p*
Total sleep time (min)	354,6 ± 131,7	404,5 ± 35,2	-0,818	n.s.
Sleep latency (min)	30,8 ± 26,3	26,2 ± 8,8	0,137	n.s.
REM latency (min)	96,5 ± 26,3	142,7 ± 55,78	-1,675	n.s.
Total waking time (min)	30,7 ± 23	16,2 ± 5	1,381	n.s.
Wake after sleep onset (min)	118,1 ± 82,2	46,2 ± 18,3	1,909	n.s.
Sleep efficiency	69,3 ± 23	83,8 ± 5	-1,381	n.s.
% Stage 1	8,6 ± 3,1	5,1 ± 2,6	1,892	n.s.
% Stage 2	59,3 ± 10,8	49,8 ± 5,3	1,763	n.s.
% Stage 3	14,7 ± 9,4	23,5 ± 7,6	-1,614	0,03*
% REM	17,4 ± 8,2	21,5 ± 2,7	-1,061	n.s.

Table 8.2 Sleep macrostructural differences between the two groups. *Significant values at $p < 0.05$

8.3.2 Spindle Analysis

An initial analysis was performed for spindle density, duration and integrated activity for the whole night. The topography of each parameter was similar between groups, with peaks in prefrontal and centroparietal areas. No statistically significant difference in slow / fast spindle duration, density or integrated activity was observed between the two groups.

8.4. Brief Discussion

Preliminary findings seem to suggest that the spindle abnormalities observed in SCZ and confirmed – to a lesser extent – by my own work in healthy FDRs, are not clearly detectable in FEP patients. Enrolled subjects had a mean duration of untreated psychosis of about 2 months (9,8 ± 1,5 weeks) and had never been treated with antipsychotics or other psychotropic compounds. At first glance, the lack of significant difference in spindle activity between the two groups appears to support the view that thalamic / thalamocortical dysfunction in SCZ is related to chronicity or neurodegenerative aspects of the disorder rather than constitutive features that can be identified when symptoms first emerge. Indeed, three studies on antipsychotic-naïve patients with SCZ reported normal spindle density during N2 sleep. Although small, sample sizes were larger (11 for Poulin et al., 2003, 8 for Forest et al., 2007, 10 for Guénole et al., 2014). Unlike the present study, spindles were counted manually in the other studies. One recent study employing an automated algorithm for spindle detection reported spindle density and amplitude deficits in 15 newly diagnosed, antipsychotic-naïve

patients with a mean age of 28 ± 8 years (Manoach et al., 2014). The authors conclude their findings implicate thalamocortical circuit dysfunction before the onset of illness, in line with the recent evidence of reduced bilateral thalamic volume correlated with sleep disturbance in UHR adolescents (Lunsford-Avery et al., 2013).

PART IV

CONCLUSIVE REMARKS

AND FUTURE PERSPECTIVES

9. TOWARDS A MOLECULAR CHARACTERIZATION OF THE SLEEP SPINDLE DEFICIT ENDOPHENOTYPE

Sleep spindles could represent a highly unspecific marker of aberrant thalamocortical oscillatory activity that is shared by severe neurodevelopmental/neurodegenerative disorders (Manoach et al., 2015). Indeed, abnormal sleep spindle activity has been reported in many clinical conditions associated with cognitive impairment, from mental retardation (Shibagaki et al., 1982), phenylketonuria (De Giorgis et al., 1986), Williams syndrome (Bodizs et al., 2012) and autism (Tessier et al., 2015) to Parkinson's disease with dementia (Latreille et al., 2015). However, spindle activity seems to adequately differentiate patients with SCZ from other patients with typical late-adolescence to young-adulthood onset disorders in psychiatry (Ferrarelli et al., 2010, Manoach, 2014). Progression of knowledge in this field will lead to a more profound understanding of the relationship between thalamocortical neurophysiology and complex interactions of cognitive, emotional and behavioural function.

The work presented here appears to support the view that impaired spindle activity is an endophenotype of SCZ rather than a biomarker of the disorder. Furthermore, Slow Wave Activity must be accurately characterized in FDRs in order to clarify whether the mild impairment of spindle activity reflects an intermediate thalamic dysfunction or a peripheral dysfunction of neocortical activity. Preliminary findings in FEP patients must be interpreted cautiously

given the small sample and warrant immediate investigation in a large group of subjects.

At the cellular level, the major sleep spindle pacemaker identified in the thalamus is $Ca_v3.3$, a voltage-dependent, T type Ca^{2+} channel (Astori et al., 2011). Calcium channels control the rapid entry of Ca^{2+} into a variety of cell types and are therefore involved in both electrical and cellular signaling. T-type channels are activated by small membrane depolarizations and are known to generate burst firing and pacemaker activity. Intriguingly, the largest GWAS of SZ risk reported common variants in *CACNA1I* – a gene located on chromosome 12 in humans – which encodes for the alpha-1 subunit of $Ca_v3.3$ (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014).

Several line of evidence currently converge on the implication of PV+ neurons in SCZ. Post-mortem studies revealed GABAergic alterations, in particular in PV+ and calretin neurons in SCZ patients. Moreover, abnormal amplitude and synchrony of oscillatory activity, mainly frontal and at high (gamma) frequencies, have been found in SCZ, during task-related, spontaneous neuronal activity (Uhlhaas and Singer, 2013), as well as after transcranial magnetic stimulation (Ferrarelli et al., 2012; Rogasch et al., 2014). Gamma frequency (30–80Hz) oscillations require the synchronized inhibition of neighboring populations of pyramidal neurons by the subclass of cortical PV+ GABAergic interneurons (Sohal et al., 2009). Given the possibility of reprogramming neural cells from induced Pluripotent Stem Cells (iPSCs), future perspectives include the need to study Calcium channel physiology in PV+

GABAergic cells reprogrammed from subjects with shared CACNA1I genetic variants. This approach will foster an in vivo characterization of the molecular bases of sleep spindle impairment that could in turn lead to novel therapeutic targets for SCZ.

Despite the use of conventional classificatory systems such as the DSM to identifiy experimental populations, the theoretical framework in which my work is set can be bridged with the Research Domain Criteria (RDoC) proposed by the National Institute of Mental Health of the United States. Indeed, RDoC promote a shift in research design from diagnostic categories towards dimensions or systems, which are supported by neuroscience and can be the basis for objective measures of psychopathology (Insel and Cuthbert, 2015). Endophenotypes are uniquely informative in traditional diagnosis-based as well as emerging RDoC contexts, “offering a bridge between the two approaches to psychopathology classification and research” (Braff, 2015).

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